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(54) Title: METHODS OF IDENTIFYING BIOLOGICAL AGENT COMPOSITIONS

(57) Abstract

New methods of identifying biological agent compositions involving (a) preparing a plurality of segmented copolymers, the segmented copolymers differing in at least one of the following, i) at least one of their segment lengths, ii) chemical structure, iii) copolymer architecture; (b) preparing compositions of the segmented copolymers with a biological agent; (c) testing at least one of the compositions of segmented copolymers with a biological agent for biological properties using a cell, animal, plant or other biological model, or measurement of a chemical or physical property in a test tube, or a theoretical model; and (d) identifying the compositions with desired biological properties.

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METHODS OF IDENTIFYING BIOLOGICAL AGENT COMPOSITIONS

This application claims the benefit of U.S. provisional application no. 60/055,256 filed August 8, 1998.

FIELD OF THE INVENTION

The present invention relates to novel methods of identifying biological agent compositions useful in pharmaceutical, biopharmaceutical, diagnostic, imaging, immunology, veterinary, and agricultural applications.

BACKGROUND OF THE INVENTION

The conventional design of a new drug is very difficult. It demands design or discovery of a new molecule which precisely matches its molecular target. Moreover, once such a molecule is discovered, the new drug candidate must be soluble, bioavailable, resistant to metabolic enzymes, and be nontoxic to the patient. Modifications of the new molecule, necessary to satisfy the above requirements, too often negatively affect its therapeutic efficacy. Due to enormous complexity, producing a new drug takes a very long time and requires huge financial resources.

Recent advances in combinatorial chemistry technologies have allowed for faster throughput in the design of new molecules. This development markedly reduces the time and cost in designing a desired molecule. However, the problem of modifying such a molecule so that it is soluble, bioavailable, resistant to metabolic enzymes, and capable of penetrating through membranes, often remains unsolved.

The drug delivery industry has addressed some of these problems, and as a result developed the ability to simplify new product development by incorporating drugs into a carrier. For drug delivery assisted products, the time of development is shortened to seven years, and the average cost is brought down significantly. Unfortunately, most drug delivery systems have several serious limitations. First,

they are able to solve only a limited number of aforementioned problems, and second they are not applicable to many drugs.

SUMMARY OF THE INVENTION

This invention is in the area of "combinatorial drug delivery" or "combinatorial formulation". The invention provides for a method of identifying a biological agent composition that can be applied to pharmaceutics, biopharmaceutics, diagnostics and imaging, immunology, veterinary, agriculture, and other areas where the properties of biological agents exhibited during interaction with a living organism or cell can be improved. Many biological agents are suitable, including those useful for diagnostics or imaging, or those that can act on a cell, organ or organism to create a change in the functioning of the cell, organ or organism. This includes, but is not limited to, pharmaceutical agents, genes, vaccines, herbicides and the like.

The current invention provides a method of identifying a biological agent composition of choice to create a complex that will render a target molecule soluble, bioavailable, resistant to metabolic enzymes, non-toxic, and freely traveling through membranes and into cells. By using segmented copolymers, such as for example, block copolymers, and preparing libraries of biological agent compositions, the invention has the ability to rapidly complex and identify the compositions of biological agents with desired biological properties. This invention can be applied in combination with high throughput screening of actual composition libraries, and can utilize mathematical concepts, which have been found to be beneficial in Combinatorial Chemistry.

The invention reduces the time and cost for creating desired drug compounds, which are not only immediately ready for clinical trials, but also possess a number of important characteristics increasing the probability of the ultimate success. Unlike combinatorial chemistry, the invention does not discover new drug structures or alter the desirable drug's characteristics, but instead provides optimal compositions of a

desired drug, solving the drug's problems of solubility, bioavailability, resistance to metabolic enzymes, toxicity, membrane transport, site specific delivery, and the like. Using a biological agent molecule as a starting point, the invention identifies new compositions with characteristics sought for the optimal performance of the selected molecule.

The invention thus relates to new methods of identifying biological agent compositions. The process involves preparing a plurality of compositions having segmented copolymers, wherein these segmented copolymers differ in at least one of their segment lengths, and then screening these compositions for the desired biological property or properties.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 shows identification of a biological agent composition with the desired (maximal) biological property (BP) using a library prepared based upon A_nB_m copolymers.

Figure 2 shows the relationship between CMC and pyrene partitioning coefficient, P, for pluronic block copolymers having varying lengths of ethylene oxide and propylene oxide segments for Pluronic L121, L101, L127, L123, L104, F108, L81, P85, P84, L61, L64, F87, L31, and F68 (37°C).

Figure 3 is a schematic of a high throughput screening procedure exemplifying the relationship between computerized analysis ("computations") using a virtual base of polymer segments ("blocks") and segmented copolymers ("carrier molecules"), chemical synthesis of new perspective carrier molecules, preparing library of biological agent compositions ("complexing"), screening selected promising compositions using physicochemical and biological analysis and identification of a biological agent composition with desired properties.

Figure 4 shows a cycle of identifying a biological agent composition of choice through the use of a parent database of carriers, computational analysis, chemical synthesis, preparation and testing of a real library carriers (base of copolymers) and biological agent compositions, and identification of a composition with desired properties ("optimized formulation").

The schematic presentations in Figure 3 and 4 serve only as examples of possible screening and identification procedures pursuant to the invention. Particularly, the elements and sequence of steps of these procedures can be varied to accommodate the properties of a certain biological agent, drug candidate and/or drug delivery situation.

DETAILED DESCRIPTION OF THE INVENTION

Definitions

•Analysis: The review and classification of data obtained from testing the compositions using high throughput screening (or otherwise) to draw conclusions from the classified data. Analysis identifies the compositions with desired biological properties answering to a set of criteria including but not limited to the following: (*i*) whether any of compositions are good enough to be a final product, and (*ii*) whether the data from the testing supports creation of a new library for a new testing cycle.

•Architecture: Refers to copolymers having the same or similar formula, but with different methods of joining each of the polymer segments.

•Basis of copolymers: A plurality of segmented copolymers differing in at least one of their segment lengths, molecular architecture, or chemical structure.

•Biological agent: An agent that is useful for diagnosing or imaging or that can act on a cell, organ or organism, including but not limited to drugs (*i.e.*, pharmaceuticals) to create a change in the functioning of the cell, organ or organism. Such agents can include, but are not limited to nucleic acids, polynucleotides,

antibacterial agents, antiviral agents, antifungal agents, anti-parasitic agents, tumoricidal or anti-cancer agents, proteins, toxins, enzymes, hormones, neurotransmitters, glycoproteins, immunoglobulins, immunomodulators, dyes, radiolabels, radio-opaque compounds, fluorescent compounds, polysaccharides, cell receptor binding molecules, anti-inflammatories, anti-glaucomic agents, mydriatic compounds, and local anesthetics.

•Biological property: Any property of a biological agent or biological agent composition that affects the action of the biological agent or biological agent composition during interaction with a biological system. This includes solubility, stability, analysis of spectral properties, binding with plasma proteins, DNA, RNA, specific receptors, enzymes or other molecules, resistance to metabolic enzymes, chemical stability, toxicity, membrane transport, of transport into, out of, within and through target cells, tissues or organs, site specific delivery, specific enzymatic activities, activation or suppression of gene expression, total DNA, RNA and protein biosynthesis, cell proliferation and differentiation, apoptosis, hormone and polypeptide secretion, bioavailability, pharmacokinetics, pharmacodynamics, efficacy, toxicity, therapeutic index and the like.

•Carrier: Segmented copolymers, and mixtures thereof including mixtures with other segmented copolymers, homopolymers, biological agents, and surfactants.

•Computational analysis: A computer program which analyzes structures of new carriers in a virtual library, predicts their interaction with the drug candidate, and selects the most promising carriers.

•Drug candidate: A substance with biological activity potentially useful for therapy. For the new composition development, the drug candidate can be used as a chemical substance or theoretical model defining the molecular structure and known properties including physicochemical properties and biological activity, mechanism

of action, disease target, initial screening results, and known or expected problems with pharmaceutical application.

•High throughput screening: Use of the set of analytical methods and procedures to test the properties of the library of the biological agent compositions. This includes screening of the composition using a biological model, for example, cell, animal or plant, measurement of physicochemical property, computational analysis, and the like.

•Library of biological agent compositions: A plurality of compositions of biological agents with carriers.

•Parent database of carriers: A computer database containing information on known drug carriers which includes (but is not limited to) at least one of the following: structure of the carrier molecules (segmented copolymers), structure and properties of its building blocks (segments), molecular architecture, and available data on properties of compositions of these carriers with various molecules, including physicochemical properties and biological activity, mechanism of action, disease target, initial screening results, and known or expected problems with pharmaceutical application.

•Preparing composition: Creation of compositions of a biological agent including drug candidates with a carrier. This includes mixing of the biological agent and the carrier under specific conditions of solvent composition, concentration, pH, temperature and the like as well as creation of computer database including parent database of carriers and information on biological agent including but not limited to chemical structure. This database can also contain available data on properties of biological agents, such as physicochemical properties, biological activity, and mechanism of action, disease target, initial screening results, and known or expected problems with pharmaceutical application.

•Segmented copolymer: A conjugate of at least two different polymer segments.

•Surfactant: A surface active agent that is adsorbed at interface.

•Testing composition: Evaluation of the properties of composition using a biological model, including but not limited to cell, animal or plant models, measurement of physicochemical property of composition, and computational analysis.

•Virtual library: A list of carriers potentially useful to the drug candidate.

The invention allows rapid selection and design of a carrier to meet specific delivery and efficacy criteria. The invention may incorporate the use of, (*i*) parental databases having a large number of chemical templates, (*ii*) exploratory virtual libraries or carriers, (*iii*) computational analysis for predicting chemical and physical properties of the complexed compounds, (*iv*) validated solid-phase and solution-phase chemistries, and (*v*) testing.

In one embodiment, the invention relates to methods of identifying biological agent compositions of choice comprising:

(a) preparing a plurality of segmented copolymers, the segmented copolymers differing in at least one of the following, (*i*) at least one of their segment lengths, (*ii*) chemical structure, (*iii*) copolymer architecture;

(b) preparing compositions of the segmented copolymers with at least one biological agent;

(c) testing at least one of the compositions of segmented copolymers with a biological agent for biological properties using a cell, animal, plant or other biological model, or measurement of a chemical or physical property in a test tube, or a theoretical model; and

(d) identifying the compositions with desired biological properties.

In another preferred embodiment, the segmented copolymer has at least one hydrophilic nonionic polymer and at least one hydrophobic nonionic segment. In another preferred embodiment, the segmented polymers have at least one cationic segment and at least one nonionic segment. In yet another preferred embodiment, the segmented polymers have at least one anionic segment and at least one nonionic segment. Also preferred are compositions where the segmented polymers have at least one polynucleotide segment and at least one segment which is not a nucleic acid. Further preferred are compositions where the polymer segments comprise at least one polypeptide segment and at least one non-peptide polymer segment.

The biological agent is an agent which is useful for diagnostics or imaging or that can act on a cell, organ or organism to create a change in the functioning of the cell, organ or organism. This includes, but is not limited to, pharmaceutical agents, genes, vaccines, herbicides and the like.

The term "preparing" is used in the broad sense to include design of theoretical models for computational analysis. The invention does not require that all or even any of segmented copolymers are synthesized, or that all or any of pharmaceutical compositions are actually prepared. The plurality of segmented copolymers can be constructed "on paper" and then tested in a computer database. Similarly, the compositions of the segmented copolymers with biological agents can be presented as a database.

The "testing" can be done using a variety of computational methods, so that part of or all process of the identification can be carried out or simulated virtually (*i.e.*, by computer).

The term "basis" refers to the plurality of segmented copolymers used in accordance with the invention.

The ability of segmented copolymers to form micelles, capture and release biological agents, interact with various systems of cell and organism affecting biological properties or otherwise interact modifying the biological response with respect to a certain biological agent depends upon the lengths (number of repeating units) in the segmented copolymer.

Within a "basis" of segmented copolymers, it has been discovered that there will be at least one copolymer which will form a composition with a biological agent exhibiting the desired biological properties. Because of the complex relationship between the biological properties and biological agent compositions, the discovery and optimization of useful biological agent compositions is ordinarily a very time-consuming process, requiring a high number of trials. The present invention provides a rational combinatorial method for identifying a useful biological agent composition by determining which copolymer of the basis possesses the desired properties with a specific biological agent.

In a preferred embodiment, the segmented copolymers have two segments having chemically different repeating units, designated "A" type and "B" type. The length of the A-type segment is designated as "*n*" and the length of B-type segment is "*m*". The length of the segment can be defined as its molecular mass or polymerization degree, or number of repeating units, atoms or the like. For example, if the length of the B-type segment is determined as a molecular mass of this segment, M_b, then the length of the A-type segment is calculated as follows:

$$M_a = M_b \frac{100-P}{P} \qquad (1)$$

wherein *P* is the weight percentage of B-type segment in the block copolymer.

Assuming for simplicity that *m* and *n* designate the number of repeating units of the B- and A-type segments, and *m* and *n* can vary from 1 to *N* independently of each

other, this will produce a base of N_2 segmented copolymers having a general formula A_nB_m. This base can be presented as a plurality of points in the 2-dimensional (2D) coordinate system $\{n, m\}$.

In accordance with the present invention, the library of biological agent compositions is tested for a useful biological property ("BP"). The BP can be any biological property including a drug therapeutic index, protein expression for a gene, immune response for a vaccine, or the like. The plurality of biological properties of the compositions within a composition library can be presented as a plurality of points in a three-dimensional space defined by a three-dimensional (3D) coordinate system $\{n, m, BP\}$. The multi-dimensional space corresponding to the library of biological agent compositions and certain biological property is termed herein as "the composition space." For example, using the base of A_nB_m diblock copolymers, the plurality of biological properties represent a three-dimensional network. The problem of identifying the optimal (for example maximal) biological parameter by testing the composition library is in essence equivalent to the mathematical problem of determining the extremum on a network (see Figure 1).

Computational and semi-computational methods have been developed to solve such problems (see, for example, G.J. Borse, *Numerical Methods With Matlab: A Resource for Scientists and Engineers* (1997); A. Dolan, J. Aldous., *Networks and Algorithms: An Introductory Approach* (1994). These methods provide for "rational testing", so that the number of compositions that have to be tested to identify the desired biological property is substantially less that the total number of compositions in the library. For example, in the composition library on the base of A_nB_m copolymers the total number of compositions equals N^2. The number of tests to be performed with this library to identify optimal biological agent composition approximates N. Using data from independent experiments, which are collectively called herein the "clues", one can further decrease the number of the tests to be performed.

The segmented copolymers of the present invention can contain more than two segments. The lengths of the copolymer segments and the BP will provide variables in the composition space. For a base of copolymers having k-segments, the composition space is presented as follows: $\{n_1, n_2 \ldots n_k, BP\}$. Similarly, several segmented copolymers with different architecture can be used in one library. In this case, the type of copolymer architecture can provide an additional variable in the composition space. For example, if j-types of the block copolymer architectures defined as Γ_j are used, then the composition space is as follows: $\{n_1, n_2 \ldots n_k, \Gamma_j, BP\}$. The identification of the useful biological agent composition of the present invention may require testing the compositions for more than one biological property. If one different biological property is to be tested, the composition space is as follows: $\{n_1, n_2 \ldots n_k, \Gamma_j, BP_1, BP_2 \ldots BP_l\}$. Concentrations of segmented copolymers and biological agents in the library of biological compositions can be also varied. In this case the concentrations of the copolymer, c_c, biological agent, c_b, provide additional variables in the composition space: $\{n_1, n_2 \ldots n_k, \Gamma_j, c_c, c_b, BP_1, BP_2 \ldots BP_l\}$.

In another preferred embodiment, the biological agent compositions contain cationic, anionic or nonionic surfactants. In such cases, the concentrations of surfactant, (c_s) provide additional variables in the composition space: $\{n_1, n_2 \ldots n_k, \Gamma_j, c_c, c_s, c_b, BP_1, BP_2 \ldots BP_l\}$. Instead of the concentration of the surfactant, the ratio of concentration of surfactants and segmented copolymers or similar dependent parameters can be used. The library can be designed using a set of homologous surfactants, for example, these differing in the length of hydrophobic groups, so that certain compositions in the library will differ from each other by type of the surfactant used. One example includes fatty acid soaps of saturated and unsaturated fatty acids. The block copolymer basis can be designed with fatty acids having varying lengths of the hydrocarbon tail (ϕ), degree of saturation (Δ), and position of unsaturated bonds (γ). All of these parameters will be additional variables in the composition space: $\{n_1, n_2 \ldots n_k, \Gamma_j, \phi, \Delta, \gamma, c_c, c_s, c_b, BP_1, BP_2 \ldots BP_l\}$.

The composition space can be designed using parameters that are dependent on the parameters characterizing the copolymer base and composition library, such as (n_1, n_2 ...n_k, Γ_j, ϕ, Δ, γ, c_c, c_s, c_b). It its believed, for example, that the critical micelle concentration (CMC) of certain amphiphilic segmented copolymers, such as poly(ethylene oxide)-block-poly(propylene oxide)-block-poly(ethylene oxide) block copolymers (also known under a name Pluronics™), depends upon the length of polymer segments (See Kabanov, *et al.*, *Macromolecules*, 28:2303-2314, 1995). Similarly, the partitioning coefficient of a drug in the block copolymer micelles, P, in certain cases depends on the length of the segments of this block copolymer (Kabanov, *et al.*, *Macromolecules*, 28:2303-2314, 1995). The relationship between CMC and P is shown in Figure 2.

In some cases, it is beneficial to use such dependent parameters as CMC and P to design the composition space. One example is a library of compositions of a pharmaceutical drug (*e.g.*, anticancer antibiotic, neuroleptic, anti-HIV drug or the like) with Pluronic block copolymers. In this case the copolymer concentration, *c*, serves as third useful parameter in the composition space. In the current example the concentration of Pluronic copolymers is varied from about 0.000001% (w/v) to about 10% (w/v) or the solubility limit of the given copolymer at 37°C. Therefore the composition space can be presented as: {CMC, P, c, BP}, where BP is a useful biological property (such as the therapeutic index).

Without wishing to be bound to a specific theory, one can exemplify the use of CMC and P in the composition space using Pluronic block copolymers. Pluronic micelles play an important role in certain biological agent compositions (Kabanov *et al.* FEBS Lett 258:343, 1989). Those micelles surround a drug with a biologically inert polymer shell, protect it while in the blood stream, deliver it to a target cell and dispense it to the target's intracellular compartment. In this way, the non-targeted cells are protected from the drug's potentially toxic effects (Kabanov *et al.*, *J. Contr. Release*, 22:141, 1992). At the same time, the single chains of Pluronic block

copolymers (so-called "unimers") inhibit certain drug efflux mechanisms resulting in increased cytotoxic activity of the drugs against multiple drug resistant (MDR) tumors (Alakhov *et al.* Bioconjugate Chemistry 7: 209 (1996); Venne *et al.*, *Cancer Research*, 56:3626, (1996)). Similar mechanisms underlie the effects of Pluronic copolymers on drug transport across the blood-brain barrier and intestinal epithelium. By changing the lengths of the ethylene(oxide) and propylene(oxide) segments, one can select Pluronic copolymers which are more effective or less effective with respect to certain classes of cells and drug transport systems.

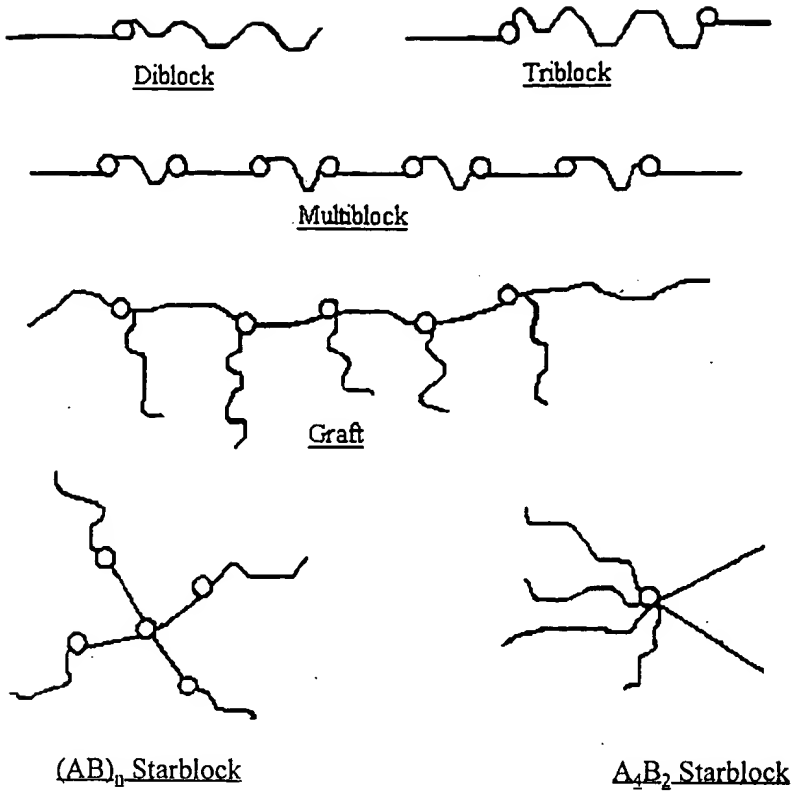
Therefore, the effects of block copolymers in biological agent compositions are two-fold. First, they form self-assembled drug carriers masking the drug from the undesired interactions in the body and providing for site specific drug delivery. Second, they act as modifiers of biological response with respect to a drug by affecting drug transport systems in the cell. These effects of block copolymer are related to the CMC and P.

Lower CMC and higher P indicate: (a) more stable micelles, (b) less block copolymer unimers available in solution, and (c) stronger attachment of the drug to micelle. These conditions correspond to the highest degrees of protection of the drug by the micelle from degradation and elimination by the body defense mechanisms. Also, the metabolism of the drug is minimal under these conditions and the drug toxic effects are minimized. However, the release of the drug from the micelles in the disease site is also minimal, thereby decreasing drug therapeutic effect. The concentration of the copolymer unimers is very low, which decreases the effects of the biological agent composition.

In contrast, when the CMCs are high and P is low, the micelles are very unstable, the concentration of unimers is high, and the drug is easily released in a free form. At the same time, high CMC is usually observed with hydrophilic block copolymers, which are also not active with respect to the drug transport systems. Further, since the drug is mainly in free form, it is also not protected from metabolic degradation,

and is more toxic than micelle-incorporated drugs. Therefore, with certain applications (for example anticancer antibiotics), selection of the optimal block copolymer composition yields Pluronic copolymers with intermediate CMC and P values. The CMC vs. P graph in Figure 2 provides a useful tool in the identification of such compositions with Pluronic block copolymers. It simplifies testing and identification procedures by using CMC vs. P isotherm instead of multidimensional space with the lengths copolymer segments as the coordinates. The information about the effects of the unimers and the micelles in drug actions provides the clues permitting to simplify the identification of the useful biological agent composition. Normally the clues are used to decrease the number of coordinates in the composition space as well as the number of compositions to be tested.

Segmented copolymers. The segmented copolymers of this invention are most simply defined as conjugates of at least two different polymer segments (see for example, Tirrel, *Interactions of Surfactants with Polymers and Proteins*, Goddard and Anantha-padmanabhan, Eds., pp. 59 *et seq.*, CRC Press, Boca Raton, Ann Arbor, London, Tokyo, 1992). Some segmented copolymer architectures are presented below:



(The straight and wavy lines designate different polymer segments while the circles designate the links between these segments.)

The simplest segmented copolymer architecture contains two segments joined at their termini to give an A-B type diblock. Conjugation of more than two segments by their termini yields an A-B-A type triblock, ...ABAB... type multiblock, or even multisegment ...ABC... architectures. If a main chain in the segmented copolymer can be defined in which one or several repeating units are linked to different polymer segments, then the copolymer have a graft architecture, *e.g.*, $A(B)_n$ type. More complex architectures include for example $(AB)_n$ or A_nB_m starblocks that have more than two polymer segments linked to a single center.

One method to produce segmented copolymers includes anionic polymerization with sequential addition of two monomers. See for example, Schmolka J., *Am. Oil*

Chem. Soc., 1977, 54:110; Wilczek-Vera *et al.*, *Macromolecules*, 1996, 29:4036. This technique yields block copolymers with a narrow molecular mass distribution of the polymeric segments. Solid-phase synthesis of block copolymers has been developed recently that permit controlling the growth of the polymer segments with very high precision (Vinogradov *et al.*, *Bioconjugate Chemistry*, 7:3, 1996). In some cases the block copolymers are synthesized by initiating polymerization of a polymer segment on ends of another polymer segment (Katayose and Kataoka, *Proc. Intern. Symp. Control. Rel. Bioact. Materials*, 1996, 23:899) or by conjugation of complete polymer segments (Kabanov *et al.*, *Bioconjugate Chem.*, 1995, 6:639; Wolfert *et al.*, *Human Gene Ther.*, 1996, 7:2123). Properties of block copolymers in relation to this invention are determined by (1) block copolymer architecture and (2) properties of the polymer segments. They are independent on the chemical structure of the links used for conjugation of these segments (see, *e.g.*, Tirrel In Interactions of Surfactants with Polymers and Proteins, Goddard and Ananthapadmanabhan, Eds., pp. 59 *et seq.*, CRC Press, Boca Raton, Ann Arbor, London, Tokyo, 1992; Sperling, Introduction to Physical Polymer Science, 2d edn., p. 46 *et seq.*, John Wiley & Sons, New York, 1993).

Linking can be accomplished by a number of reactions, many of which have been described generally in conjugate chemistry. These can involve a terminal hydroxyl group on one polymer segment, *e.g.*, R^5-O-(C_2H_4O)-H, in which R^5 is hydrogen or a blocking group such as alkyl, and an appropriate group on another polymer segment, the two being joined directly or indirectly; *i.e.*, through a third component. Alternatively, a terminal group can be converted to some other functional group, for example amino, which then is allowed to react either with the next polymer segment or another linking component. The linking group thus may be formed either by reactively involving a terminal group of a polymer segment or by replacing the terminal group. For example, a carboxylic acid group can be activated with N,N'-dicyclohexylcarbodiimide and then allowed to react with an amino or hydroxy group to form an amide or ether respectively. Anhydrides and acid chlorides will produce

the same links with amines and alcohols. Alcohols can be activated by carbonyldiimidazole and then linked to amines to produce urethane linkages or activated to produce ethers or esters. Alkyl halides can be converted to amines or allowed to react with an amine, diamines, alcohols, or diol. A terminal hydroxy group can be oxidized to form the corresponding aldehyde or ketone. This aldehyde or ketone is then allowed to react with a precursor carrying a terminal amino group to form an imine which, in turn, is reduced, with (for example) sodium borohydrate to form the secondary amine. See Kabanov *et al.*, *J. Controlled Release*, 22:141 (1992); *Meth. Enzymol.*, XLVII, Hirs & Timasheff, Eds., Acad. Press, 1977. The linkage thereby formed is an -NH- group, replacing the terminal hydroxyl group of the polymer segment.

Alternatively, a terminal hydroxyl group on the polymer can be allowed to react with bromoacetyl chloride to form a bromoacetyl ester which in turn is allowed to react with an amine precursor to form the -NH-CH_2-C(O)- linkage. Immobilized Enzymes, Berezin *et al.*, Eds., MGU, Moscow, 1976, *i.e.*, -NH-CH_2-C(O)-. The bromoacetyl ester of a polymer segment also can be allowed to react with a diaminoalkane of the formula NH_2-CqH_2q-NH_2 which in turn is allowed to react with an carboxy group on another polymer segment, or an activated derivative thereof such as an acid chloride or anhydride. The bromoacetyl ester also can be allowed to react with a cyanide salt to form a cyano intermediate. See, *e.g.*, Sekiguchi *et al.*, *J. Biochem.*, 85, 75 (1979); Tuengler *et al.*, Biochem. Biophys. Acta, 484, 1 (1977); Browne *et al*, BBRC, 67, 126 (1975); and Hunter *et al.*, J.A.C.S., 84, 3491 (1962). This cyano intermediate then can be converted to an imido ester, for instance by treatment with a solution of methanol and hydrogen chloride, which is allowed to reacted with a amine precursor to form a -NH-C(NH_2+)CH_2C(O)- linkage. A terminal hydroxyl group also can be allowed to react with 1,1'-carbonyl-bis-imidazole and this intermediate in turn allowed to react with an amino precursor to form a -NH-C(O)O- linkage. See Bartling *et al.*, Nature, 243:342 (1973).

A terminal hydroxyl also can be allowed to react with a cyclic anhydride such as succinic anhydride to yield a half-ester which, in turn, is allowed to react with a precursor having terminal amonogroup using conventional condensation techniques for forming peptide bonds such as dicyclohexylcarbodiimide, diphenylchlorophosphonate, or 2-chloro-4,6-dimethoxy-1,3,5-triazine. See *e.g.*, Means *et al.*, Chemical Modification of Proteins, Holden-Day (1971). Thus formed is the -NHC(O)-$(CH_2)_q$C(O)O- linkage.

A terminal hydroxyl group also can be allowed to react with 1,4-butanediol diglycidyl ether to form an intermediate having a terminal epoxide function linked to the polymer through an ether bond. The terminal epoxide function, in turn, is allowed to react with an amino precursor. Pitha *et al.*, *Eur. J. Biochem.*, 94:11 (1979); Elling and Kula, *Biotech. Appl. Biochem.*, 13:354 (1991); Stark and Holmberg, *Biotech. Bioeng.*, 34:942 (1989).

Halogenation of a terminal hydroxyl group permits subsequent reaction with an alkanediamine such as 1,6-hexanediamine. The resulting product then is allowed to react with carbon disulfide in the presence of potassium hydroxide, followed by the addition of proprionyl chloride to generate a isothiocyanate which in turn is allowed to react with an amino precursor to yield a -N-C(S)-N-$(CH_2)_6$-NH- linkage. See Means *et al.*, Chemical Modification of Proteins, Holden-Day (1971). The polymer chain terminating in an amino group also can be treated with phosgene and then another polymer segment containing amino group to form a urea linkage. See Means *et al.*, Chemical Modification of Proteins, Holden-Day (1971).

The polymer segment terminating in an amino group also can be treated with dimethyl ester of an alkane dicarboxylic acid and the product allowed to react with an amino precursor to produce a -N-C(NH_2+)-$(CH_2)_4$-C(NH_2+)-N- linkage. See Lowe *et al.*, Affinity Chromatography, Wiley & Sons (1974). The polymer segment terminating in an amino group can also be allowed to react with an alkanoic acid or fluorinated alkanoic acid, preferably an activated derivative thereof such as an acid

chloride or anhydride, to form a linking group -CONH-. Alternatively, an amino precursor can be treated with an α,ω-diisocyanoalkane to produce a -NC(O)NH(CH_2)$_6$NHC(O)-N- linkage. See Means, Chemical Modification of Proteins, Holden-Day (1971). Some linking groups thus can simply involve a simple functional group while others may have a spacer unit such as a polymethylene chain between two functional groups. When the linking group has such a polymethylene chain, it can have as few as two methylene units but preferably contains more; *e.g.*, six or more methylene units. The above descriptions exemplify typical strategies for the formation of linkages between the segments of the copolymers. These procedures parallel those which are known to form conjugates of biologically active agents and other agents, including the general conjugation methods described by Means *et al.*, Chemical Modification of Proteins, Holden-Day (1971); Glazer *et al.*, Chemical Modification of Proteins, Elsevier, New York (1975); Immunotechnology Catalog & Handbook, Pierce Chemical Co.; and Polyethylene Glycol Derivatives Catalog, Shearwater Polymers, Inc. (1994). It also will be appreciated that linkages which are not symmetrical, such as -CONH- or -NHCOO-, can be present in the reverse orientation; *e.g.*, -NHCO- and -OCONH-, respectively.

The polymeric segments of the copolymers can be nonionic water-soluble, nonionic hydrophobic or poorly water soluble, cationic, anionic or polyampholite, such as a polypeptide. It is preferred that the degrees of polymerization of these polymer segments independently from each other are from about 3 to about 500,000 more preferably from about 5 to about 5000, still more preferably from about 20 to about 500. If more than one segment of the same type has one segmented copolymer, then these segments may all have the same lengths or may have different lengths.

In a preferred embodiment, at least one segment of the copolymer is a nontoxic and non-immunogenic polymer which is soluble in water. Such segments include (but not are limited to) polyethers (*e.g.*, polyethylene oxide), polysaccharides (*e.g.*, dextran), polyglycerol, homopolymers and copolymers of vinyl monomers (*e.g.*,

polyacrylamide, polyacrylic esters (*e.g.*, polyacryloyl morpholine), polymethacrylamide, poly(N-(2-hydroxypropyl)methacrylamide, polyvinyl alcohol, polyvinyl pyrrolidone, polyvinyltriazole, N-oxide of polyvinylpyridine, copolymer of vinylpyridine and vinylpyridine N-oxide) polyortho esters, polyaminoacids, polyglycerols (*e.g.*, poly-2-methyl-2-oxazoline, poly-2-ethyl-2-oxazoline) and copolymers and derivatives thereof.

Preferred nonionic hydrophobic and poorly water soluble segments include polypropylene oxide, copolymers of polyethylene oxide and polyethylene oxide, polyalkylene oxide other than polyethylene oxide and polypropylene oxide, homopolymers and copolymers of styrene (*e.g.*, polystyrene), homopolymers and copolymers isoprene (*e.g.*, polyisoprene), homopolymers and copolymers butadiene (*e.g.*, polybutadiene), homopolymers and copolymers propylene (*e.g.*, polypropylene), homopolymers and copolymers ethylene (*e.g.*, polyethylene), homopolymers and copolymers of hydrophobic aminoacids and derivatives of aminoacids (*e.g.*, alanine, valine, isoleucine, leucine, norleucine, phenylalanine, tyrosine, tryptophan, threonine, proline, cistein, methionone, serine, glutamine, aparagine), homopolymers and copolymers of nucleic acid and derivatives thereof.

Preferred polyanion segments include those such as polymethacrylic acid and its salts, polyacrylic acid and its salts, copolymers of methacrylic acid and its salts, copolymers of acrylic acid and its salts, heparin, polyphosphate, homopolymers and copolymers of anionic aminoacids (*e.g.*, glutamic acid, aspartic acid), polymalic acid, polylactic acid, polynucleotides, carboxylated dextran, and the like.

Preferred polycation segments include polylysine, polyasparagine, homopolymers and copolymers of cationic aminoacids (*e.g.*, lysine, arginine, histidine), alkanolamine esters of polymethacrylic acid (*e.g.*, poly-(dimethylammonioethyl methacrylate), polyamines (*e.g.*, spermine, polyspermine, polyethyleneimine, polypropyleneimine, polybutileneimine, poolypentyleneimine, polyhexyleneimine and copolymers thereof), copolymers of tertiary amines and

secondary amines, partially or completely quaternized amines, polyvinyl pyridine and the quaternary ammonium salts of the polycation segments. These preferred polycation segments also include aliphatic, heterocyclic or aromatic ionenes (Rembaum *et al.*, Polymer letters, 1968, 6;159; Tsutsui, T., In Development in ionic polymers -2, Wilson A.D. and Prosser, H.J. (eds.) Applied Science Publishers, London, new York, vol. 2, pp. 167-187, 1986).

Additionally, dendrimers, for example, polyamidoamines of various generations (Tomalia *et al.*, Angew. Chem., Int. Ed. Engl. 1990, 29, 138) can be also used as to design the base of copolymers for combinatorial drug delivery in accordance with the current invention.

Particularly preferred are copolymers selected from the following polymer groups:

(a) segmented copolymers having at least one hydrophilic nonionic polymer and at least one hydrophobic nonionic segment ("hydrophilic-hydrophobic copolymers");

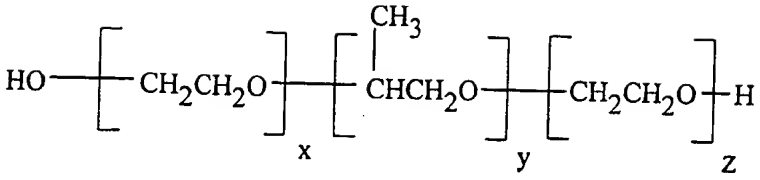
(b) segmented copolymers having at least one cationic segment and at least one nonionic segment ("cationic copolymers");

(c) segmented copolymers having at least one anionic segment and at least one nonionic segment ("anionic copolymers");

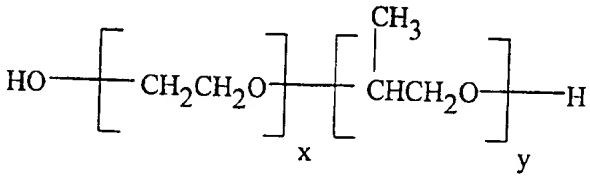
(d) segmented copolymers having at least one polypetide segment and at least one non-peptide segment ("polypeptide copolymers");

(e) segmented copolymers having at least one polynucleotide segment and at least one segment which is not a nucleic acid "polypeptide copolymers");

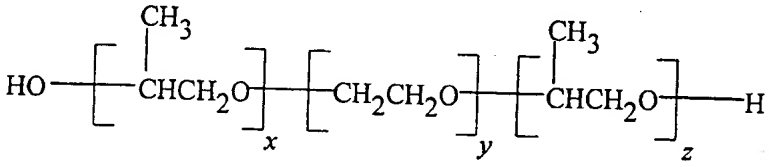
Hydrophilic-hydrophobic copolymers. Typical representatives of hydrophilic-hydrophobic copolymers are the block copolymers of ethylene oxide and propylene oxide having the formulas:



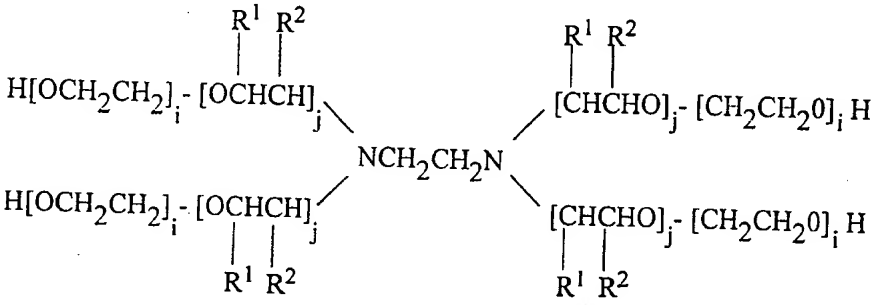
(I)



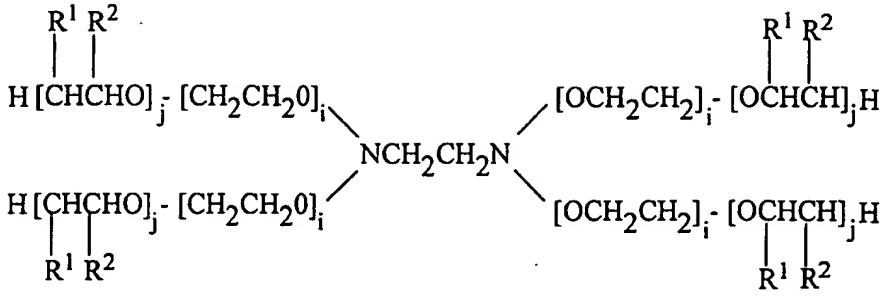
(II)



(III)



(IV)



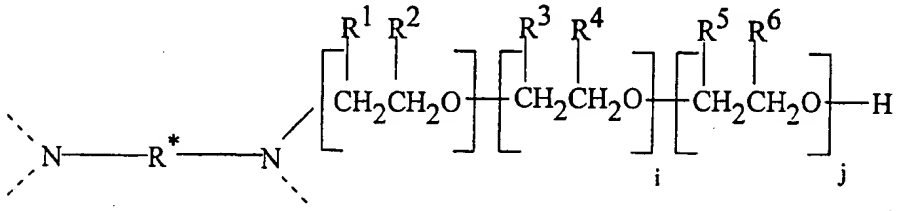
(IV-A)

in which x, y, z, i and j have values from about 2 to about 800, preferably from about 5 to about 200, more preferably from about 5 to about 80, and wherein for each R^1, R^2 pair, one is hydrogen and the other is a methyl group.

Formulas (I) through (III) are oversimplified in that, in practice, the orientation of the isopropylene radicals within the B block will be random. This random orientation is indicated in formula (IV), which is more complete. Such poly(oxyethylene)-poly(oxypropylene) compounds have been described by Santon, *Am. Perfumer Cosmet.*, 72(4):54-58 (1958); Schmolka, Loc. cit. 82(7):25 (1967); Schick, Non-ionic Surfactants, pp. 300-371 (Dekker, NY, 1967). A number of such compounds are commercially available under such generic trade names as "poloxamers", "pluronics" and "synperonics." Pluronic polymers within the B-A-B formula are often referred to as "reversed" pluronics, "Pluronic-R" or "meroxapol." The "polyoxamine" polymer of formula (XVII) is available from BASF (Wyandotte, MI) under the tradename Tetronic™. The order of the polyoxyethylene and polyoxypropylene blocks represented in formula (XVII) can be reversed, creating Tetronic-R™, also available from BASF. See, Schmolka, *J. Am. Oil Soc.*, 59:110 (1979). Polyoxypropylene-polyoxyethylene block copolymers can also be designed with hydrophilic blocks comprising a random mix of ethylene oxide and propylene oxide repeating units. To maintain the hydrophilic character of the block, ethylene oxide will predominate. Similarly, the hydrophobic block can be a mixture of ethylene oxide and propylene

oxide repeating units. Such block copolymers are available from BASF under the tradename Pluradot™.

The diamine-linked pluronic of formula (IV) can also be a member of the family of diamine-linked polyoxyethylene-polyoxypropylene polymers of formula:



(V)

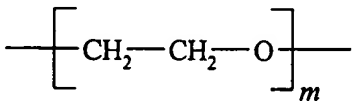
wherein the dashed lines represent symmetrical copies of the polyether extending off the second nitrogen, R* is an alkylene of 2 to 6 carbons, a cycloalkylene of 5 to 8 carbons or phenylene, for R^1 and R^2, either (a) both are hydrogen or (b) one is hydrogen and the other is methyl, for R^3 and R^4 either (a) both are hydrogen or (b) one is hydrogen and the other is methyl, if both of R^3 and R^4 are hydrogen, then one R^5 and R^6 is hydrogen and the other is methyl, and if one of R^3 and R^4 is methyl, then both of R^5 and R^6 are hydrogen.

Those of ordinary skill in the art will recognize, in light of the discussion herein, that even when the practice of the invention is confined for example, to poly(oxyethylene)-poly(oxypropylene) compounds, the above exemplary formulas are too confining. Thus, the units making up the first block need not consist solely of ethylene oxide. Similarly, not all of the B-type block need consist solely of propylene oxide units. Instead, the blocks can incorporate monomers other than those defined in formulas (I)-(V), so long as the parameters of the first embodiment are maintained. Thus, in the simplest of examples, at least one of the monomers in block A might be substituted with a side chain group as previously described.

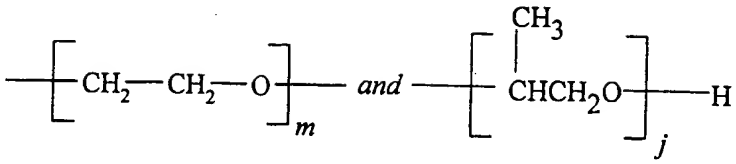
A variety of other examples of hydrophilic-hyrophobic block copolymers have been synthesized that can be used in the present invention. These copolymers have the general formula A_nB_m, wherein A is the hydrophilic homopolymer or copolymer segment, and B is a hydrophobic homopolymer or copolymer segment. Each of the A and B segments can be either straight chain or branched. Examples of block copolymers that are particularly useful in the current invention include, but are not limited to poly(ethylene oxide)-b-poly(isoprene)-b-poly(ethylene oxide) triblock copolymer (Morgan, *et al.*, *Biochem. Soc. Trans.*, 18:1021, 1990), poly(ethylene oxide)-b-poly(styrene) block copolymer (Dunn, *et al.*, *Pharm. Res.*, 11:1016, 1994), poly(ethylene oxide)-b-poly(D,L-lactide) diblock copolymer (Hagan, *et al.* Langmuir 12:2153, 1996), and poly(ethylene oxide)-b-poly((-benzyl L-aspartate) diblock copolymer (Kwon, *et al.* Langmuir 12:945, 1993).

The hydrophilic homopolymer or copolymer A segments in hydrophilic-hyrophobic block copolymers that can be used in the present invention will contain at least three monomeric units, each of which unit will have the same or different pendant group. Each pendant group will contain at least one atom selected from the group consisting of oxygen and nitrogen. Representative hydrophilic homopolymers or copolymers include but are not limited to polyethylene oxides, copolymers of ethylene oxide and propylene oxide, polysaccharides, polyacrylamides, polygycerols, polyvinylalcohols, polyvinylpyrrolidones, polyvinylpyridine N-oxides, copolymers of vinylpyridine N-oxide and vinylpyridine, polyoxazolines, and polyacroylmorpholines.

Preferably, the hydrophilic A segment is:



a copolymer of



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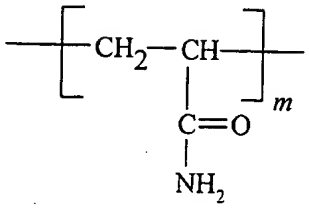

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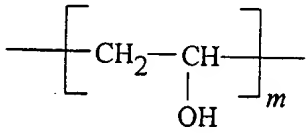

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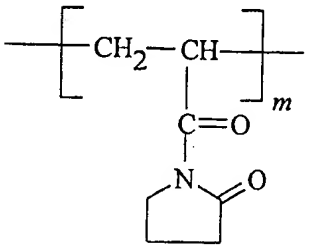

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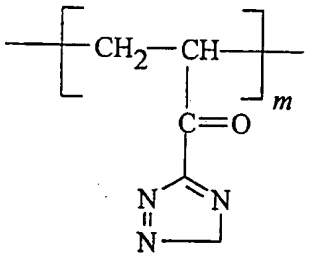

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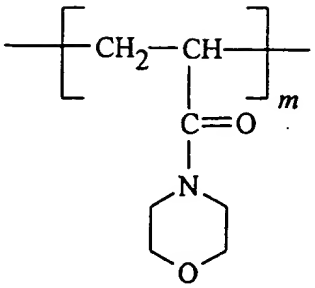
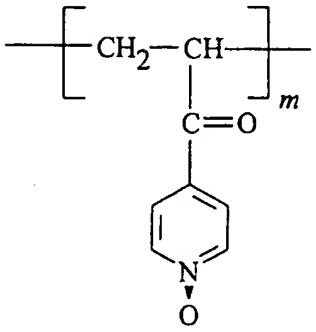


or



in which each of *m* and *j* has a value of from 3 to 5000.

The hydrophobic B segments useful in this invention can also contain fluorocarbon moieties including but not limited to fluoroalkyl segments, and copolymers containing both fluorocarbon and hydrocarbon. One such example is the segmented block copolymers having the formula:

$$R^1\text{-}L^1\text{-}\{R^2\text{-}L^2\text{-}A\}w\text{-}L^4\text{-}R^4\text{-}L^3\text{-}R^3$$

(VI)

in which:

either, (i) R^1 is a monovalent fluorinated hydrocarbon of 2 to 50 carbon atoms and R^2 is a divalent hydrocarbon of 2 to 50 carbon atoms or (ii) R^1 is a monovalent

hydrocarbon of 2 to 50 carbon atoms and R^2 is a divalent fluorinated hydrocarbon of 2 to 50 carbon atoms;

R^3 is, (i) hydrogen, (ii) a monovalent fluorinated hydrocarbon of 2 to 50 carbon atoms, or (iii) a monovalent hydrocarbon of 2 to 50 carbon atoms;

R^4 is, (i) a bond if R^3 is hydrogen; (ii) a divalent hydrocarbon of 2 to 50 carbon atoms if R^3 is a fluorinated hydrocarbon, or (iii) a divalent fluorinated hydrocarbon of 2 to 50 carbon atoms if R^3 is a hydrocarbon;

each of L^1 and L^2, independently of the other, is a linking group;

L^3 and L^4 taken together with R^4, is a bond if R^3 is hydrogen or if R^3 is other than hydrogen each of L^3 and L^4, taken independently is a linking group;

A is a hydrophilic homopolymer or copolymer comprising at least three monomeric units each having the same or different pendant group containing at least atom selected from the group consisting of oxygen and nitrogen; and

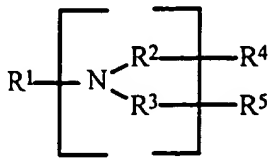
w has a value of from 1 to 100.

The hydrophilic homopolymer or copolymer A will contain at least three monomeric units, each of which unit will have the same or different pendant group. Each pendant group will contain at least one atom selected from the group consisting of oxygen and nitrogen. Representative hydrophilic homopolymers or copolymers include polyethylene oxides, copolymers of ethylene oxide and propylene oxide, polysaccharides, polyacrylamides, polygycerols, polyvinylalcohols, polyvinylpyrrolidones, polyvinylpyridine N-oxides, copolymers of vinylpyridine N-oxide and vinylpyridine, polyoxazolines, and polyacroylmorpholines.

Cationic copolymers. Useful segmented copolymers include a class of copolymers in which at least one segment is a polycation. One example of these structures is a basis of copolymers comprising a plurality of covalently bound

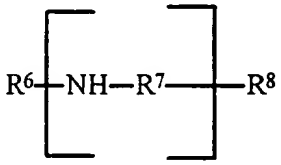
polymer segments wherein the segments have (a) at least one polycation segment which segment is a cationic homopolymer, copolymer, or block copolymer comprising at least three aminoalkylene monomers, the monomers being selected from the group consisting of at least one of the following:

(*i*) at least one tertiary amino monomer of the formula:



A.

and the quaternary salts of the tertiary amino monomer, or (ii) at least one secondary amino monomer of the formula:



B.

and the acid addition and quaternary salts of the secondary amino monomer, in which:

R^1 is hydrogen, alkyl of 2 to 8 carbon atoms, an A monomer, or a B monomer; each of R^2 and R^3, taken independently of the other, is the same or different straight or branched chain alkanediyl group of the formula:

$$—(C_zH_{2z})—$$

in which z has a value of from 2 to 8; R^4 is hydrogen satisfying one bond of the depicted geminally bonded carbon atom; and R^5 is hydrogen, alkyl of 2 to 8 carbon atoms, an A monomer, or a B monomer; R^6 is hydrogen, alkyl of 2 to 8 carbon atoms, an A monomer, or a B monomer; R^7 is a straight or branched chain alkanediyl group of the formula:

$$—(C_zH_{2z})—$$

in which z has a value of from 2 to 8; and R^8 is hydrogen, alkyl of 2 to 8 carbon atoms, an A monomer, or a B monomer; and

(b) at least one straight or branched nonionic hydrophilic segment A having from about 5 to about 1000 monomeric units which is defined above.

The polycationic segments in the copolymers of the invention can be branched. For example, polyspermine-based copolymers are branched. The cationic segment of these copolymers was synthesized by condensation of 1,4-dibromobutane and N-(3-aminopropyl)-1,3-propanediamine. This reaction yields highly branched polymer products with primary, secondary, and tertiary amines.

An example of branched polycations are products of the condensation reactions between polyamines containing at least 2 nitrogen atoms and alkyl halides containing at least 2 halide atoms (including bromide or chloride). In particular, the branched polycations are produced as a result of polycondensation. An example of this reaction is the reaction between N-(3-aminiopropyl)-1,3-propanediamine and 1,4-dibromobutane, producing polyspermine.

Another example of a branched polycation is polyethyleneimine represented by the formula:

$$(NHCH_2CH_2)_x [N(CH_2CH_2)CH_2CH_2]_y$$

(VI)

One example of useful polyamine-based copolymers is the polymer of formula:

$$K^1\text{-}L^1\text{-}[G\text{-}L^2\text{-}F\text{-}L^3]l\text{-}K^2,$$

(VII)

in which:

F is a polyamine segment comprising a plurality of repeating units of formula -NH-R^0, wherein R^0 is an aliphatic group of 2 to 6 carbon atoms, which may be substituted;

G is polyethylene oxide or copolymer ethylene oxide and propylene oxide a straight or branched nonionic segment defined above;

K^1 and K^2 independently of the other, is hydrogen, hydroxy group, amonogroup, G or F polymer segments;

and each of L^1, L^2 and L^3, independently of the other, is a linking group or chemical bond.

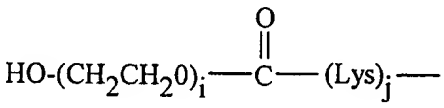
The amino groups of the polycationic segments can be quaternized to produce ammonium salts. Examples include polyspermine and polyamines that are modified with alkylhalides to produce tertiary and quaternized polyamines. Another useful type of cationic segments of well defined chemical structure are ionenes that can be aliphatic, heterocyclic or aromatic (Rembaum *et al.* Polymer Letters, 1968, 6:159;

Tsutsui, T., Development in ionic polymers, Wilson, A.D. and Prosser, H.J. (eds.), Applied Science Publishers, London, New York, vol. 2, pp. 163-187, 1986).

Anionic copolymers. Anionic copolymers contain at least one polyelectrolyte segment that yields a polyanion in an aqueous environment. This includes both strong polyacids having high ionization degrees in a broad range of pH, and weak polyacids characterized by pH-dependent ionization degrees. Anionic segments normally have a plurality of pendant amino groups such as carobxylic groups, sulfate groups, sulfonate groups, phosphate groups, and the like. Examples of anionic copolymers include but are not limited to polyoxyethylene-b-polymethacrylic acid (Wang, *et al.*, *J. Polym. Sci.*, Part A: Polym. Chem., 30:2251, 1992), polystyrene-b-polyacrylic acid (Zhong, *et al.* Macromolecules, 25:7160, 1992), polyacrylic acid grafted with polyoxyethylene-b-polyoxypropylene-b-polyoxyethylene (Bromberg and Levin, Macromol. Rapid Commun. 17:169 1996).

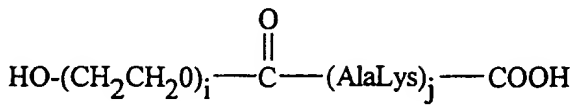
Polypeptide copolymers. Polypeptide copolymers have a plurality of covalently bound polymer segments wherein the segments have at least one polypeptide segment and at least one non-peptide polymer segment. Polypeptide segments have a plurality of amino acid units or derivatives thereof.

Examples of useful segmented copolymers containing polypeptides include the poly(oxyethylene)-poly-L-lysine) diblock copolymer of the following formula:



(XVIII)

wherein *i* is an integer of from about 2 to about 500, and *j* is an integer from about 4 to about 500. A second example is the poly(oxyethylene)-poly-(L-alanine-L-lysine) diblock copolymer of formula:



(XIX)

wherein *i* is an integer of from about 2 to about 500, and *j* is an integer from about 2 to about 500.

The use of polypeptide copolymers in the invention allows for better control of the polypeptide segment lengths by using solid-phase and solution-phase chemistries. Segmented copolymers based on polypeptides with well defined chemical structures have been described in the literature, such as poly(amino acid)-b-poly(N,N-dietylacrylamide)-b-poly(amino acid) (Bromberg and Levin, Bioconjugate Chem. 9:40, 1998). Further, the unit composition and sequence in polypeptides can be varied including hydrophobic, hydrophilic, ionizable, hydrogen and chemical bond forming amino acids and derivatives thereof to produce broader variability in the basis of the segmented copolymers.

Polynucleotide copolymers. Polynucleotide copolymers have a plurality of covalently bound polymer segments wherein the segments have at least one segment containing at least three nucleic acid units or the derivatives thereof. Similar to polypeptide copolymers, the polynucleotide copolymers provide for better control over segment length and sequence by using solid-phase and solution-phase chemistries. Segmented copolymers based on polynucleotides with well-defined chemical structure have been described including, polyoxyethylene-b-polynucleotide copolymer and polycation-b-polynucleotide copolymer (Vinogradov *et al.*, Bioconjugate Chemistry, 7:3, 1996; See also U.S. Patent No. 5,656,611). As with polypeptide copolymers, polynucleotide copolymers permit variation of the unit

composition and sequence in polynucleotide segments which is particularly useful in selecting proper biological agent compositions pursuant to this invention.

Screening assays and composition identification. The screening assays of this invention are analytical tests which are useful in characterizing and selecting biological agent compositions by sorting them for "positive" and "negative" compositions according to the initially defined criteria. Normally, one or more screening assays are required to identify a preferable biological agent composition.

Depending upon the task, varieties of *in vitro* cell-free and cell-based, as well as *in* vivo screening assays can be used to select preferred biological agent compositions. This includes, but is not limited to, physico-chemical tests such as the characterization of biological agent solubility and stability, analysis of spectral properties; characterization of biological agent binding with plasma proteins, DNA, RNA, specific receptors, enzymes or other molecules; transport-related tests such as analyses of transport into, out of, within, and through target cells, tissues or organs; functional tests such as analyses of specific enzymatic activities, activation or suppression of gene expression, total DNA, RNA and protein biosynthesis, cell proliferation and differentiation assays, apoptosis analysis, hormone and polypeptide secretion assays; in vivo pharmacological tests, such as pharmacokineticts, pharmacodynamics of biological agent, its efficacy, toxicity and therapeutic index.

Since libraries of biological agent compositions are screened in this invention, high-throughput and ultra-high-throughput screening assays are preferred. Depending on the specific properties of the biological agent, and obstacles related to the properties that need to resolved by using present invention, various combinations of the screening assays can be used to identify a biological agent composition.

Many biological agents have limited solubility in aqueous solutions, therefore, they often cannot be administered at required doses in the body without specifically selected and optimized delivery systems for improving biological agent solubility. In

one embodiment, libraries of biological agent compositions with segmented copolymer carriers are generated and screened to improve biological agent solubility in aqueous solutions. In the related screening system, solubility of the biological agent alone is determined and compared to that of a biological agent formulated with the selected carriers. A variety of methods can be used to determine the biological agent solubility including but not limiting light absorption, fluorescent, spectrophothometry, circular dichroism, calorimetry, NMR, ESR, chromatography; mass spectrometry and the like.

One of the most common problems related to the limited performance of biological agents is their insufficient stability, which is often related to their high sensitivity to metabolic enzymes. Such enzymes, depending on the biological agent structure, include proteases, nucleases, redox enzymes, transferases, etc. In one preferred embodiment, libraries of biological agent compositions with segmented copolymer carriers are generated and screened to protect biological agent from degradation by metabolic enzymes. The screening can include treatment of the biological agent with isolated enzymes, their combinations or enzymatic complexes existing in isolated fractions of cells or tissues, followed by analysis of the native biological agent level in the analyzed sample. The screening can also be based on the biological agent administration in a whole organism followed by sampling and analysis of the native biological agent level in the sample, or by continuous monitoring of the native biological agent level in the body. Verity of methods could be used for detection of the native biological agent level including but not limiting HPLC, LC-MS, GC-MS, radioisotope methods, NMR, various bioassays, etc.

Another common obstacle that limits biological agent effectiveness is insufficient circulation time in the body due to clearance of the biological agent by the reticuloendothelial system. According to the present invention, the libraries of biological agent compositions with segmented copolymer carriers can be prepared and screened to reduce the biological agent clearance. The screening methods can be

based upon direct measurement of the biological agent binding with serum proteins such as albumin, low density and ultralow density lipopolyproteins, and the like. Also, these screening methods can use the analysis of the biological agent phagocytosis by isolated cell populations such as macrophages, polymorphonulear cells, etc. The screening can also be based on biological agent administration in a whole organism followed by sampling and analysis of the native biological agent level in the sample, or by continuos monitoring of the native biological agent level in the body. Combinations of the above procedures can also be used.

Reduced efficacy of the biological agent is also often caused by its low bioavailability. For example, most polypeptides and proteins, polynucleotides, as well as many low molecular weight pharmaceutical drugs are not effective when administered orally. An important factor that limits oral bioavailability of the above pharmaceutical agents is their reduced adsorption through small intestinal epithelial tissue. According to the present invention, the libraries of such biological agents with segmented copolymer carriers can be prepared and screened to increase biological agent oral bioavailability. The screening methods include, but are not limited to the measurement of the biological agent transport across polarized epithelial cell monolayers, for example, Caco-2 or Caco-4 cell monolayers. Another bioavailability-related problem is low efficacy of central nervous system agents, which caused by limited transport of the agents across brain microvessel endothelial tissue that is also known as blood brain barrier (BBB). Libraries of biological agent compositions with segmented copolymer carriers can be generated and screened for compositions that increase biological agent transport across BBB. The screening methods for this assay can be based on the measurement of the biological agent transport across polarized endothelial cell monolayers, such as primary bovine brain microvessel endothelial cells (BBMEC), human primary and immortalized brain microvessel endothelial cells, etc. The bioavailability screening procedures also include administration of the biological agent in a whole organism followed by sampling and analysis of the native biological agent level in the sample, or by

continuos monitoring of the native biological agent level in the body. Combinations of the above procedures can also be used. Depending on the nature of the biological agent, the efficacy of its transport can be measured by various methods including but not limited to fluorescence, absorption or other spectroscopy, radioisotope methods, various bio- or immunoassays, etc.

Biological activity of many biological agents is often significantly reduced due to insufficient efficacy of the biological agent transport through the cell membrane. To resolve this problem, libraries of biological agent compositions with segmented copolymer carriers can be generated and screened for compositions that can improve biological agent transmembraneous properties. Verity of screening methods can be used to evaluate the efficacy of biological agent transport through the membrane. These methods include but not limited by those based on analysis of the biological agent transport across artificial membranes such as lipid bilayers and liposomes, analysis of the biological agent uptake in cells including, cell lines, primary cell cultures, bacterial strains and isolates, etc. Depending on the biological agent nature, the efficacy of its transport can be measured by various methods including but not limited fluorescent, absorption or other spectroscopy, radioisotope methods, various bio- or immunoassays, etc.

The libraries of biological agent composition can be also screened using direct measurement of the biological agent biological activity. Depending on the biological agent properties, verity of screening methods can be used to analyze biological agent biological effect. For example, for many anticancer agents apoptosis assays, such as TONEL staining; proliferation assays, such as thymidine DNA incorporation rate analysis, MTT, XTT and colony formation assays can be used to evaluate efficacy of the selected compositions. Cell adhesion based methods could be used for evaluation of immune modulating compositions.

Depending on the nature of the biological agent, more specific screening methods could be used to evaluate efficacy of the biological agent compositions including but

not limiting analyses of activity of specific enzymes that are known to be direct or indirect targets for particular biological agent; analysis of signal transduction events (such as tyrosine phosphorylation, association or dissociation of SH2 and/or SH3 signaling proteins, changes in second messenger levels, etc.) that are involved in the biological agent mechanism of action; analyses of specific gene expression (by using hybridization, RT-PCR and/or protein expression assays) that known to involved in the biological agent mechanism of action.

In general, any assay that relates to biological activity, transport, pharmacokinetics, stability of a biological agent compositions can be used to screen libraries of biological agent compositions with segmented copolymer carriers for the best performing composition with preselected biological agent.

The screening and identification of the biological agent compositions pursuant this invention can use a virtual library. Without wishing to be limited to any particular computational analysis, the use of the virtual library is exemplified as follows. The starting information for identifying the desired biological agent composition includes, (i) the drug structure, (ii) the database of available polymeric segments for synthesis of segmented copolymers, and (iii) accumulated data on segmented copolymer carriers, if available. The starting data also includes the selection of desirable biological properties, which identify the preferred biological agent composition for the given biological agent including drug candidates. New carrier molecules are virtually assembled by combining structures of polymeric segments stored in computer database. The combination is subjected to the rules of the bond formation as if a new compound is synthesized as a result of a chemical reaction yielding a segmented copolymer of a specific molecular architecture. A plurality of virtually designed segmented copolymers is a virtual copolymer base. The physicochemical and biological properties of each copolymer of this base is then predicted in relation to its specific architecture using the properties of separate blocks and or model segmented copolymers that were actually synthesized and characterized

in the experiment. The properties of the isolated segments include but are not limited to chemical structures of the repeating units, lengths of polymeric segments, QSAR parameters derived from the structures and physicochemical and biological parameters available from experiments or derived from experimental data. Exemplary properties of segments include molecular weight, volume, surface, hydrophobicity, hydration energy, partitioning coefficients, ionization degree (for polyelectrolytes), etc. The prediction of the copolymer property can be based on any known or expected relationship between the properties of the segments and the copolymer property.

Without wishing to be limited to a particulate theory, it is believed that interpolation or extrapolation of any previously accumulated data on properties of copolymers gives the predicted values. At this stage, the data on the experimentally determined biological properties of the compositions of the biological agent, for example, drug candidate, and actually synthesized model segmented copolymers are used in the computerized analysis. The predicted properties of the copolymers and their compositions with biological agents are then compared to the desired values, and the score of fit is calculated. Finally, the virtual carrier molecules from the base are classified according their score, and the best compositions are identified.

Without wishing to be limited by a particular theory, it is believed that possible elements in the identification of a biological agent composition with desired properties include but are not limited to parent databases comprising a large variety of chemical templates, exploratory virtual library of carriers, computational analysis predicting chemical and physical properties of biological agent compositions, validated segmented copolymer chemistries (including solid-phase and solution-phase chemistries) and high throughput screening.

Biological agents. The biological agents of the invention are agents useful for diagnostics or imaging, or those that can act on a cell, organ or organism to create a change in the functioning of the cell, organ or organism, including but not limited to

pharmaceutical drugs, immunoadjuvants, vaccines genes, herbicides and the like. Such biological agents are used in *e.g.*, diagnostics, therapy, immunization or otherwise are applied to combat human and animal disease. Such agents include but are not limited to nucleic acids, polynucleotides, antibacterial agents, antiviral agents, antifungal agents, antiparasitic agents, tumoricidal or anti-cancer agents, proteins, toxins, enzymes, hormones, neurotransmitters, glycoproteins, immunoglobulins, immunomodulators, dyes, radiolabels, radio-opaque compounds, fluorescent compounds, polysaccharides, cell receptor binding molecules, anti-inflammatories, anti-glaucomic agents, mydriatic compounds and local anesthetics.

Special classes of biological agents that can be used in this invention include pharmaceutical drugs. Many drugs are rapidly cleared from the body or are degraded by the body's defense mechanisms. These problems, plus toxic side effects, seriously limit drug efficacy by reducing time available to the drug to reach its target and by limiting the amount of drug which can safely be given to the patient. In addition, many drugs do not readily penetrate tissues or effectively seek out and concentrate in appropriate cells to maximize their therapeutic effect. The use of the biological agent compositions pursuant to this invention permits to significantly improve therapeutic drugs by decreasing in their side effects, and increase in therapeutic action.

The biological agents with which the present compositions can be used include but are not limited to non-steroidal anti-inflammatories such as indomethacin, salicylic acid acetate, ibuprofen, sulindac, piroxicam, and naproxen, antiglaucomic agents such as timolol or pilocarpine, neurotransmitters such as acetylcholine, anesthetics such as dibucaine, neuroleptics such as the phenothiazines (for example compazine, thorazine, promazine, chlorpromazine, acepromazine, aminopromazine, perazine, prochlorperazine, trifluoperazine, and thioproperazine), rauwolfia alkaloids (for example, resperine and deserpine), thioxanthenes (for example chlorprothixene and tiotixene), butyrophenones (for example haloperidol, moperone, trifluoperidol, timiperone, and droperidol), diphenylbutylpiperidines (for example pimozde), and

benzamides (for example sulpiride and tiapride); tranquilizers such as glycerol derivatives (for example mephenesin and methocarbamol), propanediols (for example meprobamate), diphenylmethane derivatives (for example orphenadrine, benzotrapine, and hydroxyzine), and benzodiazepines (for example chlordiazepoxide and diazepam); hypnotics (for example zolpdem and butoctamide); beta-blockers (for example propranolol, acebutonol, metoprolol, and pindolol); antidepressants such as dibenzazepines (for example, imipramine), dibenzocycloheptenes (for example, amtiriptyline), and the tetracyclics (for example, mianserine); MAO inhibitors (for example phenelzine, iproniazid, and selegeline); psychostimulants such as phenylehtylamine derivatives (for example amphetamines, dexamphetamines, fenproporex, phentermine, amfeprramone, and pemoline) and dimethylaminoethanols (for example clofenciclan, cyprodenate, aminorex, and mazindol); GABA-mimetics (for example, progabide); alkaloids (for example codergocrine, dihydroergocristine, and vincamine); anti-Parkinsonism agents (for example L-dopamine and selegeline); agents utilized in the treatment of Altzheimer's disease, cholinergics (for example citicoline and physostigmine); vasodilators (for example pentoxifyline); and cerebro active agents (for example pyritinol and meclofenoxate). These agents include also DNA topoisomerase inhibitors (including type I and type II), brain and tumor imaging agents, free radical scavenger drugs, anticoagulants, ionotropic drugs, and neuropeptides such as endorphins.

The biological agent compositions also can be used advantageously with anti-neoplastic agents such as paclitaxel, daunorubicin, doxorubicin, carminomycin, 4'-epiadriamycin, 4-demethoxy-daunomycin, 11-deoxydaunorubicin, 13-deoxydaunorubicin, adriamycin-14-benzoate, adriamycin-14-actanoate, adriamycin-14-naphthaleneacetate, vinblastine, vincristine, mitomycin C, N-methyl mitomycin C, bleomycin A2, dideazatetrahydrofolic acid, aminopterin, methotrexate, cholchicine and cisplatin, antibacterial agents such as aminoglycosides including gentamicin, antiviral compounds such as rifampicin, 3'-azido-3'-deoxythymidine (AZT), and acylovir; antifungal agents such as azoles including fluconazole, macrolides such as

amphotericin B, and candicidin; anti-parastic compounds such as antimonials. These biological agents include without limitation vinca alkaloids, such as vincristine and vinblastine, mitomycin-type antibiotics, such as mitomycin C and N-methyl mitomycin, bleomycin-type antibiotics such as bleomycin A2, antifolates such as methotrexate, aminopterin, and dideaza-tetrahydrofolic acid, taxanes, anthracycline antibiotics and others.

The compositions also can utilize a variety of polypeptides such as antibodies, toxins such as diphtheria toxin, peptide hormones, such as colony stimulating factor, and tumor necrosis factors, neuropeptides, growth hormone, erythropoietin, and thyroid hormone, lipoproteins such as μ-lipoprotein, proteoglycans such as hyaluronic acid, glycoproteins such as gonadotropin hormone, immunomodulators or cytokines such as the interferons or interleukins, hormone receptors such as the estrogen receptor.

The compositions also can be used with enzyme inhibiting agents such as reverse transcriptase inhibitors, protease inhibitors, angiotensin converting enzymes, 5μ-reductase, and the like. Typical of these agents are peptide and nonpeptide structures such as finasteride, quinapril, ramipril, lisinopril, saquinavir, ritonavir, indinavir, nelfinavir, zidovudine, zalcitabine, allophenylnorstatine, kynostatin, delaviridine, bis-tetrahydrofuran ligands (see, for example Ghosh *et al.*, J. Med. Chem. 1996, 39:3278), and didanosine. Such agents can be adminitered alone or in combination therapy; *e.g.*, a combination therapy utilizing saquinavir, zalcitabine, and didanosine, zalcitabine, and zidovudine. See, for example, Collier *et al.*, Antiviral Res., 1996, 29:99.

The biological agent compositions can be used with nucleic acids such as thymine, polynucleotides such as DNA or RNA polymers or synthetic oligonucleotides, which may be derivatized by covalently modifying the 5'- or the 3'-end of the polynucleic acid molecule with hydrophobic substituents to facilitate entry into cells (see for example, Kabanov *et al.*, FEBS Lett., 1990, 259, 327; Kabanov and

Alakhov, *J. Contr. Rel.*, 1990, 28:15). Additionally, the phosphate backbone of the polynucleotides has been modified to remove the negative charge (see, for example, Agris *et al.*, *Biochemistry*, 1968, 25:6268, Cazenave and Helene in Antisense Nucleic Acids and Proteins: Fundamentals and Applications, Mol and Van der Krol., Eds., p. 47 *et seq.*, Marcel Dekker, New York, 1991), or the purine or pyrimidine bases has been modified, for example, to incorporate photo-induced crosslinking groups, alkylating, groups, organometallic groups, intercalating groups, biotin, fluorescent and radioactive groups (see, for example, Antisense Nucleic Acids and Proteins: Fundamentals and Applications, Mol and Van der Krol, Eds., p. 47 *et seq.*, Marcel Dekker, New York, 1991; Milligan *et al.* In Gene Therapy for Neoplastic Diseases, Huber and Laso, Eds. P. 228 *et seq.*, New York Academy of Sciences, New York, 1994). Such nucleic acid molecules can be among other things antisense nucleic acid molecules, phosphodiester, oligonucleotide (-anomers, ethylphospotriester analogs, phosphorothioates, phosphorodithioates, phosphoro-ethyletriesters, methylphosphonates, and the like (see, for example, Crooke, Anti-Cancer Drug Design 1991, 6:609; De Mesmaeker, *et al.*, Acc. Chem. Res. 1995, 28:366). The invention is used with antigene, ribozyme and aptamer nucleic acid drugs (see, for example, Stull and Szoka, Pharm. Res. 1995, 12:465).

Included among the suitable biological agents are viral genomes and viruses (including the lipid and protein coat). This accounts for the possibility of using a variety of viral vectors in gene delivery (*e.g.*, retroviruses, adenoviruses, herpes-virus, Pox-virus) used as complete viruses of their parts. See, for example, Hodgson, Biotechnology, 1995, 13:222.

Suitable biological agents include oxygen transporters (*e.g.*, porphines, porphirines and their complexes with metal ions), coenzymes and vitamins (*e.g.*, NAD/NADH, vitamins B12, chlorophylls), and the like.

Suitable biological agents further include agents used in diagnostics visualization methods, such as magnetic resonance imaging (*e.g.*, gadolinium (III)

diethylenetriamine penta-acetic acid), and may be a chelating group (*e.g.*, diethylenetriamine penta-acetic acid, triethylenetriamine pentaacetic acid, ethylenediamine-tetraacetic acid, 1,2-diaminocyclo-hexane-N,N,N',N'-tetra-aceticacid, N,N'-di(2-hydroxybenzyl) ethylene diamine), N-(2-hydroxyethyl) ethylene diamine triacetic acid and the like). Such biological agents may also include an alpha-, beta-, or gamma-emitting radionuclide (*e.g.*, gallium 67, indium 111, technetium 99). Suitable biological agents also include iodine containing radiopaque molecules.

The biological agent may also be a diagnostic agent, which may include a paramagnetic or superparamagnetic element, or combination of paramagnetic element and radionuclide. The paramagnetic elements include but are not limited to, gadolinium (III), dysporsium (III), holmium (III), europium (III) iron (III) or manganese (II).

The invention can be also used to identify useful fibrinolytic compositions with enzymes such as streptokinase, urokinase, tissue plasminogen activator or other fibrinolytic enzyme that is effective in dissolving blood clots and reestablishing and maintaining blood flow through trombosed coronary or other blood vessels. Also this invention is used to identify useful compositions for treating burns, circulatory diseases in which there is an acute impairment of circulation, in particular, microcirculation, respiratory distress syndrome, as well as compositions for reducing tissue damage during angioplasty procedures. Further, the compositions identified using this invention including these to treat myocardial damage, ischemic tissue, tissue damaged by reperfusion injury, stroke, sickle cell anemia and hypothermia. These compositions are especially useful for treating vascular obstructions caused by abnormal cells which is an often complication during malaria and leukemia and are suitable as a perfusion medium for transplantation of organs. The invention is also suitable for identifying the compositions of antiinfective compounds, as well as modulators of immune response, and improved adjuvants, antigens and vaccines.

Adjuvants suitable for use in this invention include but are not limited to adjuvants of mineral, bacterial, plant, synthetic or host product origin. Suitable mineral adjuvants include aluminum compounds such as aluminum particles and aluminum hydroxide. Suitable bacterial adjuvants include but are not limited to, muramyl dipeptides, lipid A, Bordetella pertussis, Freund's Complete Adjuvant, lipopolysaccharides and its various derivatives, and the like. Suitable adjuvants include without limitation small immunogenes, such as sythetic peptide of malaria, polysaccharides, proteins, bacteria and viruses. Antigens that can be used in the present invention are compounds which, when introduced into a mammal will result in formation of antibodies. Suitable antigens include but are not limited to natural, recombinant, or synthetic products derived from viruses, bacteria, fungi, parasites and other infectious agents, as well as autoimmune disease, hormones or tumor antigens used in prophylactic or therapeutic vaccines. These antigens include components produced by enzymatic cleavage or can be compounds produced by recombinant DNA technique. Suitable viral antigens include but are not limited to HIV, rotavirus, influenza, foot and mouth disease, herpes simplex, Epstein-Barr virus, Chicken pox, pseudorabies, rabies, hepatitis A, hepatitis B, hepatitis C, measles, distemper, Venezuelan equine encephalomyelitis, Rota virus, polyoma tumor virus, Feline leukemia virus, reovirus, respiratory synticial virus, Lassa fever virus, canine parvovirus, bovine pappiloma virus, tick borne encephalitis, rinderpest, human rhinovirus species, enterovirus species, Mengo virus, paramixovirus, avian infectious bronchitis virus. Suitable bacterial antigens include but are not limited to Bordetella pertussis, Brucella abortis, Escherichia coli, salmonella species, salmonella typhi, streptococci, cholera, shigella, pseudomonas, tuberculosis, leprosy and the like. Also suitable antigens include infections such as Rocky Mountain spotted fever and thyphus, parasites such as malaria, schstosomes and trypanosomes, and fungus such as Cryptococcus neoformans. The protein and peptide antigens include subunits of recombinant proteins (such as herpes simplex, Epstein Barr virus, hepatitis B, pseudorabies, flavivirus, Denge, yellow fever, Neissera gonorrhoeae, malaria, trypanosome surface antigen, alphavirus, adenovirus and the like), proteins (such as

diphteria toxoid, tetanus toxoid, meningococcal outer membrane protein, streptococcal M protein, hepatitis B, influenza hemagglutinin and the like), synthetic peptides (*e.g.* malaria, influenza, foot and mouth disease virus, hepatitis B, hepatitis C). Suitable polysaccharide and oligosaccharide antigens originate from pneumococcus, haemphilis influenza, neisseria meningitides, Pseudomonas aeruginosa, Klebsiella pneumoniae, pneumococcus.

Surfactants. Surfactants are defined herein in the most general sense as surface active agents that are adsorbed at interface (see, for example, Martin, Physical Pharmacy, 4th ed., p.370 *et seq.*, Lea & Febiger, Philadelphia, London, 1993). These surface active agents in particular decrease the surface tension at the air-water interface in aqueous solutions (see, for example, Martin, Physical Pharmacy, 4th ed., p.370 *et seq.*, Lea & Febiger, Philadelphia, London, 1993) and include without limitation micelle forming amphiphiles, soaps, lipids, surface active drugs and other surface active biological agents, and the like (see, for example, Martin, Physical Pharmacy, 4th ed., Lea & Febiger, Philadelphia, London, 1993; Marcel Dekker, New York, Basel, 1979; Atwood and Florence, *J. Pharm. Pharmacol.*, 1971, 23:242S; Atwood and Florence, *J. Pharm. Sci.*, 1974, 63:988; Florence and Attwood, Physicochemical Principles of Pharmacy, 2d. edn., p.180 *et seq.*, Chapman and Hall, New York, 1988; Hunter, Introduction to Modern Colloid Science, p.12 *et seq.*, Oxford University Press, Oxford, 1993).

Cationic surfactants that can be used in the present biological agent compositions include but are not limited to primary amines (*e.g.*, hexylamine, heptylamine, octylamine, decylamine, undecylamine, dodecylamine, pentadecyl amine, hexadecyl amine, oleylamine, stearylamine, diaminopropane, diaminobutane, diaminopentane, diaminohexane, diaminoheptane, diaminooctane, diaminononane, diaminodecane, diaminododecane), secondary amines (*e.g.*, N,N-distearylamine), tertiary amines (*e.g.*, N,N',N'-polyoxyethylene(10)-N-tallow-1,3-diaminopropane), quaternary amine salts (*e.g.*, dodecyltrimethylammonium bromide, hexadecyltrimethylammonium bromide,

alkyltrimethylammonium bromide, tetradecyltrimethylammonium bromide, benzalkonium chloride, benzethonium chloride, benzylonium bromide, benzyldimethyldodecylammonium chloride, benzyldimethylhexadecylammonium chloride, benzyltrimethylammonium methoxide, cetyldimethylethylammonium bromide, dimethyldioctadecyl ammonium bromide, methylbenzethonium chloride, decamethonium chloride, methyl mixed trialkyl ammonium chloride, methyl trioctylammonium chloride), 1,2-diacyl-3-(trimethylammonio)propane (acyl group = dimyristoyl, dipalmitoyl, distearoyl, dioleoyl), 1,2-diacyl-3-(dimethylammonio)propane (acyl group = dimyristoyl, dipalmitoyl, distearoyl, dioleoyl), 1,2-dioleoyl-3-(4'-trimethylammonio) butanoyl-sn-glycerol, 1,2-dioleoyl-3-succinyl-sn-glycerol choline ester, cholesteryl (4'-trimethylammonio) butanoate), heterocyclic amines, imidazoles, thiazolium salts, N-alkyl pyridinium and quinaldinium salts (*e.g.*, cetylpyridinium halide), N-alkylpiperidinium salts, dialkyldimetylammonium salts, dicationic bolaform electrolytes ($C_{12}Me_6$; $C_{12}Bu_6$), dialkylglycetylphosphorylcholine, lysolecithin), cholesterol hemisuccinate choline ester, lipopolyamines (*e.g.*, dioctadecylamidoglycylspermine (DOGS), dipalmitoyl phosphatidylethanolamidospermine (DPPES), N'-octadecylsperminecarboxamide hydroxytrifluoroacetate, N',N''-dioctadecylsperminecarboxamide hydroxytri-fluoroacetate, N'-nonafluoropentadecylosperminecarboxamide hydroxytrifluoroacetate, N',N''-dioctyl(sperminecarbonyl)glycinamide hydroxytrifluoroacetate, N'-(heptadecafluorodecyl)-N'-(nonafluoropentadecyl)-sperminecarbonyl)glycinamede hydroxytrifluoroacetate, N'-[3,6,9-trioxa-7-(2'-oxaeicos-11'-enyl)heptaeicos-18-enyl]sperminecarboxamide hydroxytrifluoroacetate, N'-(1,2-dioleoyl-sn-glycero-3-phosphoethanoyl)-spermine carboxamide hydroxytrifluoroacetate) (see, for example, Behr *et al.*, *Proc. Natl. Acad. Sci.* 1989, 86:6982; Remy *et al.*, Bioconjugate Chem. 1994, 5:647), 2,3-dioleyloxy-N-[2(spermine-carboxamido)ethyl]-N,N-dimethyl-1-propanaminiumtrifluoroacetate (DOSPA) (see, for example, Ciccarone *et al.*, Focus 1993, 15:80), N,N',N'',N'''-tetramethyl-N,N',N'',N'''-tetrapalmitylspermine (TM-TPS) (Lukow *et al.*, *J. Virol.*, 1993, 67:4566), N-[1-(2,3-dioleyloxy)propyl]-N,N,N-trimethylamonium chloride (DOTMA) (see, for example, Felgner, *et al.*, *Proc. Natl. Acad. Sci.*, USA

1987, 84:7413; Ciccarone *et al.*, Focus 1993, 15:80), dimethyl dioctadecylammonium bromide (DDAB) (see, for example, Whitt *et al.*, Focus 1991, 13:8), 1,2-dioleoyl-3-dimethyl-hydroxyethyl ammonium bromide (DORI) (see, for example, Felgner *et al.*, *J. Biol. Chem.*, 1994, 269:2550), 1,2-dioleyloxypropyl-3-dimethyl-hydroxyethyl ammonium bromide (DORIE) (see, for example, Felgner *et al.*, *J. Biol. Chem.* 1994, 269:2550), 1,2-dioleyloxypropyl-3-dimethyl-hydroxypropyl ammonium bromide (DORIE-HP) (see, for example, Felgner *et al.*, *J. Biol. Chem.*, 1994, 269:2550), 1,2-dioleyloxypropyl-3-dimethyl-hydroxybutyl ammonium bromide (DORIE-HB) (see, for example, Felgner *et al.*, *J. Biol. Chem.* 1994, 269:2550), 1,2-dioleyloxypropyl-3-dimethyl-hydroxypentyl ammonium bromide (DORIE-HPe) (see, for example, Felgner *et al.*, *J. Biol. Chem.*, 1994, 269:2550), 1,2-dimyristyloxypropyl-3-dimethyl-hydroxyethyl ammonium bromide (DMRIE) (see, for example, Felgner *et al.*, *J. Biol. Chem.*, 1994, 269:2550), 1,2-dipalmitoyloxypropyl-3-dimethyl-hydroxyethyl ammonium bromide (DPRIE) (see, for example, Felgner *et al.*, *J. Biol. Chem.*, 1994, 269:2550), 1,2-distearoyloxypropyl-3-dimethyl-hydroxyethyl ammonium bromide (DSRIE) (see, for example, Felgner *et al.*, *J. Biol. Chem.*, 1994, 269:2550), N,N-dimethyl-N-[2-(2-methyl-4-(1,1,3,3-tetramethylbutyl)-phenoxy]ethoxy)ethyl]-benze-nemethanaminium chloride (DEBDA), N-[1-(2,3-dioleyloxy)propyl]-N,N,N,-trimethylammonium methylsulfate (DOTAB), lipopoly-L(or D)-lysine (see, for example, Zhou, *et al.*, Biochim. Biophys. Acta 1991, 1065:8), poly(L (or D)-lysine conjugated to N-glutarylphosphatidylethanolamine lysine (see, for example, Zhou, *et al.*, Biochim. Biophys. Acta 1991, 1065:8), didodecyl glutamate ester with pendant amino group ($C_{12}GluPhCnN^+$) (see, for example, Behr, Bioconjugate Chem. 1994, 5:382), ditetradecyl glutamate ester with pendant amino group ($C_{14}GluC_nN^+$) (see, foe example, Behr, Bioconjugate Chem. 1994, 5:382), 9-(N',N''-dioctadecyl-glycinamido)acridine (see, for example, Remy *et al.*, Bioconjugate Chem. 1994, 5:647), ethyl 4-[[N-[3-bis(octadecylcarbamoyl)-2-oxapropylcarbonyl]-glycinamido]pyrrole-2-carboxamido]-4-pyrrole-2-carboxylate (see, for example, Remy *et al.*, Bioconjugate Chem. 1994, 5:647), N',N'-dioctadecylornithylglycinamide hydroptrifluoroacetate (see, for example, Remy *et al.*,

Bioconjugate Chem. 1994, 5:647), cationic derivatives of cholesterol (*e.g.*, cholesteryl-3(-oxysuccinamidoethylenetrimethylammonium salt, cholesteryl-3(-oxysuccinamidoethylenedimethylamine, cholesteryl-3(-carboxyamido-ethylenetrimethylammonium salt, cholesteryl-3(-carboxyamidoethylenedimethyl-amine, 3([N-(N',N'-dimethylaminoetane-carbomoyl] cholesterol) (see, for example, Singhal and Huang, In Gene Therapeutics, Wolff, Ed., p.118 *et seq.*, Birkhauser, Boston, 1993), pH-sensitive cationic lipids (*e.g.*, 4-(2,3-bis-palmitoyloxy-propyl)-1-methyl-1H-imidazole, 4-(2,3-bis-oleoyloxy-propyl)-1-methyl-1H-imidazole, cholesterol-(3-imidazol-1-yl propyl) carbamate, 2,3-bis-palmitoyl-propyl-pyridin-4-yl-amine) and the like (see, for example, Budker, *et al.*, *Nature Biotechnology*, 1996, 14:760).

Especially useful in the context of gene delivery and other applications are compositions with mixtures of cationic surfactant and nonionic surfactants. This includes, but is not limited to, dioloeoyl phosphatidylethanolamine (DOPE), dioleoyl phosphatidylcholine (DOPC) (see, for example, Felgner, *et al.*, *Proc. Natl. Acad. Sci.*, USA (1987); Singhal and Huang, Gene Therapeutics, Wolff, Ed., p. 118 *et seq.*, Birkhauser, Boston, 1993). This also includes commercially available cationic lipid compositions including but not limited to LipofectAMINE™, Lipofectine®, DMRIE-C, CellFICTIN™, LipofectACE™ Transfectam reagents (see, for example, Ciccarone *et al.*, Focus 1993, 15:80; Lukow *et al.*, *J. Virol.*, 1993, 67:4566; Behr, Bioconjugate Chem. 1994, 5:382; Singhal and Huang, Gene Therapeutics, Wolff, Ed., p. 118 *et seq.*, Birkhauser, Boston, 1993; GIBCO-BRL Co.; Promega Co., Sigma Co.) and other cationic lipid compositions used for transfection of cells (see, for example, Felgner *et al.*, *J. Biol. Chem.*, 1994, 269:2550; Budker, *et al.*, Nature Biotechnology 1996, 14:760).

Anionic surfactants that can be used in the biological agent compositions include but are not limited to alkyl sulfates, alkyl sulfonates, fatty acid soap including salts of saturated and unsaturated fatty acids and derivatives (*e.g.*, adrenic acid, arachidonic acid, 2-octenoic acid, octanoic acid, nonanoic acid, decanoic acid, undecanoic acid,

unde celenic acid, lauric acid, myristoleic acid, myristic acid, palmitic acid, palmitoleic acid, heptadecanoic acid, stearic acid, nonanedecanoic acid, heneicosanoic acid, docasanoic acid, tricosanoic acid, tetracosanoic acid, cis-15-tetracosenoic acid, hexacosanoic acid, heptacosanoic acid, octacosanoic acid, triocantanoic acid), salts of hydroxy-, hydroperoxy-, polyhydroxy-, epoxy- fatty acids (see, for example, Ingram and Brash, Lipids 1988, 23:340; Honn *et al.*, Prostaglandins 1992, 44:413; Yamamoto, *Free Radic. Biol. Med.*, 1991, 10:149; Fitzpatrick and Murphy, Pharmacol. Rev., 1989, 40:229; Muller *et al.*, Prostaglandins 1989, 38:635; Falgueyret *et al.*, FEBS Lett. 1990, 262:197; Cayman Chemical Co., 1994 Catalog, pp. 78-108), salts of carboxylic acids (*e.g.*, valeric acid, trans-2,4-pentadienoic acid, hexanoic acid, trans-2-hexenoic acid, trans-3-hexenoic acid, 2,6-heptadienoic acid, 6-heptenoic acid, heptanoic acid, pimelic acid, suberic acid, sebacicic acid, azelaic acid, undecanedioic acid, decanedicarboxylic acid, undecanedicarboxylic acid, dodecanedicarboxylic acid, hexadecanedioic acid, docasenedioic acid, tetracosanedioic acid, prostanoic acid and its derivatives (*e.g.*, prostaglandins) (see, for example, Nelson *et al.*, C&EN 1982, 30-44; Frolich, Prostaglandins, 1984, 27:349; Cayman Chemical Co., 1994 Catalog, pp. 26-61), leukotrienes and lipoxines (see for example, Samuelsson *et al.*, Science 1987, 237:1171; Cayman Chemical Co., 1994 Catalog, pp. 64-75), alkyl phosphates, O-phosphates (*e.g.*, benfotiamine), alkyl phosphonates, natural and synthetic lipids (*e.g.*, dimethylallyl pyrophosphate ammonium salt, S-farnesylthioacetic acid, farnesyl pyrophosphate, 2-hydroxymyristic acid, 2-fluorpalmitic acid, inositoltrphosphates, geranyl pyrophosphate, geranygeranyl pyrophosphate, (-hydroxyfarnesyl phosphonic acid, isopentyl pyrophoshate, phosphatidylserines, cardiolipines, phosphatidic acid and derivatives, lysophosphatidic acids, sphingolipids and like), synthetic analogs of lipids such as sodium-dialkyl sulfosuccinate (*e.g.*, Aerosol OT®, n-alkyl ethoxylated sulfates, n-alkyl monothiocarbonates, alkyl- and arylsulfates (asaprol, azosulfamide, p-(benzylsulfonamideo)benzoic acid, cefonicid, CHAPS), mono- and dialkyl dithiophosphates, N-alkanoyl-N–methylglucamine, perfluoroalcanoate, cholate and desoxycholate salts of bile acids, 4-chloroindoleacetic acid, cucurbic acid, jasmonic

acid, 7-epi jasmonic acid, 12-oxo-phytodienoic acid, traumatic acid, tuberonic acid, abscisic acid, acitertin, and the like.

Cationic and anionic surfactants that can be used in accordance with the invention also include fluorocarbon and mixed fluorocarbon-hydrocarbon surfactants. See, for example, Mukerjee, P. Coll. Surfaces A: Physicochem. Engin. Asp., 1994, 84: 1; Guo *et al.* J. Phys. Chem.1991, 95: 1829; Guo *et al.* J. Phys. Chem.1992, 96: 10068. The list of such surfactants that are useful in current inventions includes but is not limited to the salts of perfluorocarboxylic acids (*e.g.*, pentafluoropropionic acid, heptafluorobutyric acid, nonanfluoropentanoic acid, tridecafluoroheptanoic acid, pentadecafluorooctanoic acid, heptadecafluorononanoic acid, nonadecafluorodecanoic acid, perfluorododecanoic acid, perfluorotetradecanoic acid, hexafluoroglutaric acid, perfluoroadipic acid, perfluorosuberic acid, perfluorosebacicic acid), double tail hybrid surfactants $(C_mF_{2m+1})(C_nH_{2n+1})CH\text{-}OSO_3Na$. See, for example, Guo *et al.*, J. Phys. Chem., 1992, 96: 6738, Guo *et al.*, *J. Phys. Chem.*, 1992, 96:10068; Guo *et al.*, *J. Phys. Chem.*, 1992, 96:10068), fluoroaliphatic phosphonates, fluoroaliphatic sulphates, and the like.

The biological agent compositions may additionally contain nonionic or zwitterionic surfactants including but not limited to phospholipids (*e.g.*, phosphatidylethanolamines, phosphatidylglycerols, phosphatidylinositols, diacyl phosphatidylcholines, di-O-alkyl phosphatidylcholines, platelet-activating factors, PAF agonists and PAF antagonists, lysophosphatidylcholines, lysophosphatidyl-ethanolamines, lysophosphatidylglycerols, lysophosphatidylinositols, lyso-platelet-activating factors and analogs, and the like), saturated and unsaturated fatty acid derivatives (*e.g.*, ethyl esters, propyl esters, cholesteryl esters, coenzyme A esters, nitrophenyl esters, napthyl esters, monoglycerids, diglycerides, and triglycerides, fatty alcohols, fatty alcohol acetates, and the like), lipopolysaccharides, glyco- and shpingolipids (*e.g.*, ceramides, cerebrosides, galactosyldiglycerides, gangliosides, lactocerebrosides, lysosulfatides, psychosines, shpingomyelins, sphingosines,

sulfatides), chromophoric lipids (neutral lipids, phospholipids, cerebrosides, sphingomyelins), cholesterol and cholesterol derivatives, Amphotericin B, abamectin, acediasulfone, n-alkylphenyl polyoxyethylene ether, n-alkyl polyoxyethylene ethers (*e.g.*, Triton™, sorbitan esters (*e.g.*, Span™, polyglycol ether surfactants (Tergitol™, polyoxyethylenesorbitan (*e.g.*, Tween™, polysorbates, polyoxyethylated glycol monoethers (*e.g.*, Brij™, polyoxylethylene 9 lauryl ether, polyoxylethylene 10 ether, polyoxylethylene 10 tridecyl ether), lubrol, copolymers of ethylene oxide and propylene oxide (*e.g.*, Pluronic™, Pluronic-R™, Teronic™, Pluradot™, alkyl aryl polyether alcohol (Tyloxapol™, perfluoroalkyl polyoxylated amides, N,N-bis[3-d-gluconamidopropyl]cholamide, decanoyl-N-methylglucamide, n-decyl (-d-glucopyranozide, n-decyl (-d-glucopyranozide, n-decyl (-d-maltopyranozide, n-dodecyl (-d-glucopyranozide, n-undecyl (-d-glucopyranozide, n-heptyl (-d-glucopyranozide, n-heptyl (-d-thioglucopyranozide, n-hexyl (-d-glucopyranozide, n-nonanoyl (-d-glucopyranozide 1-monooleyl-rac-glycerol, nonanoyl-N-methylglucamide, n-dodecyl (-d-maltoside, n-dodecyl (-d-maltoside, N,N-bis[3-gluconamidepropyl]deoxycholamide, diethylene glycol monopentyl ether, digitonin, heptanoyl-N-methylglucamide, heptanoyl-N-methylglucamide, octanoyl-N-methylglucamide, n-octyl (-d-glucopyranozide, n-octyl (-d-glucopyranozide, n-octyl (-d-thiogalactopyranozide, n-octyl (-d-thioglucopyranozide, betaine ($R^1R^2R^3N+R'CO_2-$, where $R^1R^2R^3R'$ hydrocarbon chains), sulfobetaine ($R^1R^2R^3N+R'SO_3-$), phoshoplipids (*e.g.* dialkyl phosphatidylcholine), 3-[(3-cholamidopropyl)-dimethylammonio]-2-hydroxy-1-propanesulfonate, 3-[(3-cholamidopropyl)-dimethylammonio]-1-propanesulfonate, N-decyl-N,N-dimethyl-3-ammonio-1-propane-sulfonate, N-dodecyl-N,N-dimethyl-3-ammonio-1-propanesulfonate, N-hexadecyl-N,N-dimethyl-3-ammonio-1-propane-sulfonate, N-octadecyl-N,N-dimethyl-3-ammonio-1-propanesulfonate, N-octyl-N,N-dimethyl-3-ammonio-1-propane-sulfonate, N-tetradecyl-N,N-dimethyl-3-ammonio-1-propanesulfonate, dialkyl phosphatitidyl-ethanolamine.

The following examples will serve to further typify the nature of the invention but should not be construed as a limitation on the scope thereof, which is defined solely by the appended claims.

EXAMPLE 1
Testing a library of oligonucleotide compositions

A. GOAL: Identify the composition of antisense oligonucleotide against ICAM-1 designed using a base of polyamine-polyether copolymers with the following characteristics: at least 50% inhibition of ICAM-1 in IFN-γ stimulated murine endothelial cells Bend.3.

B. The 20-mer phosphorothioate antisense oligonucleotide IP-3082 (Strepkowski, *et al.*, J. Immunol. 1994) was synthesized using a DNA synthesator as previously described.

C. A base of polyamine-polyether copolymers was synthesized as previously described (Kabanov *et al.*, *Bioconjugate Chem*, 6:639-643, 1995). This base includes, 1) polyspermine (10)-b-polyoxyethylene (34) ("PS915"); 2) polyspermine (10)-b-polyoxyethylene(104) ("PS946"); 3) polyspermine(10)-b-polyoxyethylene(182) ("PS980"); 4) polyspermine(10)-b-polyoxy-ethylene(27)-b-polyoxypropylene(40)-b-polyoxyethylene(27) (PSP85); 3,4-ionene-b- polyoxy-ethylene(182) ("PEG I3,4"); polyethyleneimine(40) (PEI); polyethyleneimine(500) 25% grafted with polyoxyethylene(114) ("P50MPEI/25"); polyethyleneimine(500) 55% grafted with polyoxyethylene(114) ("P50MPEI/50"); polyethyleneimine(500) 75% grafted with polyoxyethylene(114) ("P50MPEI/75"); polyoxyethylene(182)-polyethyleneimine(40)-b-polyoxy-ethylene(182) (P80PEI/1); polyethyleneimine(500) 25 % grafted with polyoxyethylene(182) ("P80MPEI/2/25"). Here the number of repeating units of each is presented in brackets. The copolymers were formulated with 2*-mer phosphorothioate antisense oligonucleotide IP-3082 (Strepkowski, *et al.*,

J. Immunol., 1994) against ICAM-1 at polymer to oligonucleotide ratio (w/w) 1:1, 3:1, 5:1, 6:1, 10:1, 17:1 and 20:1 to form a composition library.

D. The biological activity of the library of oligonucleotide compositions was tested *in vitro* using a cell model. The test included assay of inhibition of expression of ICAM-1 using murine endothelial cells Bend.3, stimulated by IFN-(to increase expression of ICAM-1 (Strepkowski, *et al.* J. Immunol. 1994). Briefly, Bend.3 cells were maintained in culture flasks with RPMI1640 medium supplemented with 10% FBS, 1% Penicillin-Streptomycin and 1% Hepes at 37°C, 5% CO_2. Plated Bend.3 cells were treated with IP-3082 alone or with the compositions of the composition library in RPMI1640 medium without serum at a final concentration of oligonucleotide 2(M. Free oligonucleotide reveals no activity under these conditions. After 4 hours incubation at 37°C, cells were washed twice with PBS, and 25 U/ml IFN-(in RPMI1640 medium supplemented with 10% serum was added to cells followed by incubation at 37°C for 16 hours. ICAM-1 expression was evaluated by Flow Cytometry using an biotinylated antibody against murine ICAM-1 (YN1) that was revealed after with Streptavidin-Phycoerythrin conjugate. The optimal composition as determined in this test is P80PEI/2/25 at nitrogen to phosphate ratio 20:1. The results were as follows.

Composition	Inhibition of ICAM-1 at the optional nitrogen to phosphate ratio %	Optimal polymer to oligonucleotide ratio (w/w)
PS915	0	At any ratio
PS980	15%	1:1
PS946	0	At any ratio
PSP85	0	At any ratio
PEG I,3,4	0	At any ratio
PEI	27%	3:1
P50MPEI/25	40%	5:1
P50MPEI/50	40%	10:1
P50MPEI/75	33%	17:1
P80PEI/1	33%	6:1
P80PEI/2/25	60%	20:1

[1)] Mean values of triplicates are presented, SEM is less than 10% ($p<0.05$)

EXAMPLE 2
Testing a library of plasmid compositions

A. GOAL: Identify the compositions of expression vector pAPR-ICAM-1 encoding the murine ICAM-1 under the control of Cytomegalovirus (CMV) promoter from a library of cationic segmented copolymers with the following characteristics: transfection efficacy in COS-7 cells exceeds that of Lipofectamine.

B. Plasmid pAPR-ICAM-1 containing murine ICAM-1 gene under the control of the CMV promoter was propagated in E. coli DH5a strain and purified using the Flexiprep kit from Pharmacia.

C. A base of cationic, segmented copolymers consists of polyamine-polyether copolymers listed in Example 1C, polyethyleneimine(500)-b-polyoxyethylene(550) ("PE250200/2"), and polylysine-polyether conjugates synthesized as previously described (US Patent 5656611): polylysine(150) 25% grafted with

polyoxyethylene(114) ("PL18050/20"), polylysine(150) 50% grafted with polyoxyethylene(114) ("PL18050/50"), polylysine(150) 75% grafted with polyoxyethylene(114) ("PL18050/75"), polylysine(19)-b-polyoxyethylene(114) ("PL40/50"), polylysine(19)-b-polyoxyethylene(41)-b-polyoxypropylene(16)-b-polyoxyethylene(41) ("PL40/F38"), polylysine-(19)-b-polyoxyethylene(27)-b-polyoxypropylene(40)-b-polyoxyethylene-(27) ("PL40/P85"), polylysine-(19)-b-polyoxyethylene(17)-b-polyoxypropylene(64)-b-polyoxy-ethylene(17) ("PL40/P123"), polylysine(100)-b- polyoxyethylene(114) ("PL12050"). Here, the number of repeating units of each is presented in brackets. The copolymers were formulated with the expression vector pAPR-ICAM-1 at polymer to DNA ratio (w/w) 1:1, 3:1, 40:1 and 6:1, 85:1 to form a composition library.

D. Screening protocol. COS-7 cells were maintained in culture flasks with DMEM medium supplemented with 10% FBS, 1% Penicillin-Streptomycin and 1% Hepes, at 37°C, 5% CO_2. Compositions of polymer with plasmid pAPR-ICAM-1 were prepared by mixing DNA plasmid with various polymer in the medium for 20 min. at 37°C. Lipofectamine was used at the concentration of 15 mg/ml. Plated Bend.3 cells were treated with DNA alone or with the compositions in DMEM medium without serum at the final DNA concentration of 2 (g/ml. After 4 hours incubation at 37°C, cells were washed twice with PBS and DMEM medium supplemented with 10% serum was added to cells followed by an incubation at 37°C for 16 hours. ICAM-1 expression was evaluated by Flow Cytometry using an biotinylated antibody against murine ICAM-1 (YN1) that was revealed after with Streptavidin-Phycoerythrin conjugate. The results of testing are shown in the table. The desired composition as determined in this test is PE250200/2 at nitrogen to phosphate ratio 1:1. This composition surpasses Lipofectamine.

Composition tested	ICAM-1 expression, % of Lipofectamine	Number of expression, % of Lipofectamine	Optimal polymer to DNA ratio (w/w)
PS915	0	0	At any ratio
PS946	0	0	At any ratio
PS980	0	0	At any ratio
PSP85	0	0	At any ratio
PEG 13,4	0	0	At any ratio
PL18050/25	0	0	At any ratio
PL18050/50	0	0	At any ratio
PL18050/75	0	0	At any ratio
PL40/50	0	0	At any ratio
PL40/F38	0	0	At any ratio
PL40/P85	0	0	At any ratio
PL40/P123	0	0	At any ratio
PL12050	0	0	At any ratio
PEI	100%	0	3:1
P50MPEI/25	19%	50%	40:1
P50MPEI/50	18%	50%	85:1
P80PEI/1	0	0	At any ratio
P50MPEI/75	0	0	At any ratio
P80PEI/2/25	0	0	At any ratio
PE250200/2	120%	138%	1:1

EXAMPLE 3

Testing micelle-formation in Pluronic block copolymer base

A. Goal: To identify Pluronic copolymer forming most stable micelles in aqueous solutions.

B. Pluronic block copolymers were obtained from BASF Co. (Parispany, NJ) The base of Pluronic block copolymers was designed including: polyoxyethylene(1)-b-polyoxypropylene(16)-b-polyoxyethylene(1) (Pluronic L31), polyoxyethylene(2)-b-polyoxypropylene(31)-b-polyoxyethylene(2) (Pluronic L61), polyoxyethylene(13)-b-polyoxypropylene(31)-b-polyoxyethylene(13) (Pluronic L64), polyoxyethylene(80)-b-polyoxypropylene(31)-b-polyoxyethylene(80) (Pluronic F68), polyoxyethylene(3)-b-polyoxypropylene(40)-b-polyoxyethylene(3) Pluronic L81, polyoxyethylene(17)-b-

polyoxypropylene(40)-b-polyoxyethylene(17) (Pluronic P84), polyoxyethylene(27)-b-polyoxypropylene(40)-b-polyoxyethylene(27) (Pluronic P85), Pluronic F87, polyoxyethylene(4)-b-polyoxypropylene(53)-b-polyoxyethylene(4) (Pluronic L101), polyoxyethylene(23)-b-polyoxypropylene(53)-b-polyoxyethylene(23) (Pluronic L104), polyoxyethylene(136)-b-polyoxypropylene(53)-b-polyoxyethylene(136) (Pluronic F108), polyoxyethylene(5)-b-polyoxypropylene (64)-b-polyoxyethylene(5) (Pluronic L121), polyoxyethylene(17)-b-polyoxypropylene(64)-b-polyoxyethylene(17) (Pluronic L123) and polyoxyethylene(95)-b-polyoxypropylene(64)-b-polyoxyethylene(95) (Pluronic L127). Here the number of repeating units of each is presented in brackets.

C. The critical micelle concentrations (CMCs) of Pluronic copolymers of the base B were determined at 37°C, phosphate-buffered saline, pH 7.4 using pyrene fluorescent probe as previously described by Kabanov *et al.* (Macromolecules 28:2303, 1995). The results are presented in the table. Result: Pluronic L121 forms the most stable micelles (lowest CMC).

Pluronic copolymer	CMC, M
L31	1.2E-02
L61	1.1E-04
L64	4.8E-04
F68	3.0E-04
L81	2.3E-05
P84	1.7E-04
P85	6.5E-05
F87	9.0E-05
L101	2.1E-06
L104	3.3E-06
F108	2.0E-05
L121	9.0E-07
L123	4.3E-06
F127	3.0E-06

EXAMPLE 4
Testing drug solubilization in Pluronic block copolymer base

A. Goal: To identify Pluronic copolymer having the highest capacity for solubilizing a model drug, pyrene.

B. Same base of Pluronic block copolymers is used as in Example 3B.

C. The partitioning coefficients (P) of pyrene in the micelles of Pluronic copolymers were determined at 37°C, phosphate-buffered saline, pH 7.4 using pyrene fluorescent probe as previously described by Kabanov *et al.* (Macromolecules 28:2303, 1995). The results are presented in the table. Result: Pluronic forms the highest capacity for solubilizing pyrene (highest P).

Pluronic copolymer	P
L31	225
L61	4193
L64	444
F68	171
L81	2400
P84	533
P85	2000
F87	229
L101	14286
L104	6000
F108	2000
L121	20000
L123	9524
F127	9090

EXAMPLE 5
Prediction of CMC and partitioning coefficient for the Pluronic base

A. Goal: To predict CMC and P for polyoxyethylene(9)-b-polyoxypropylene(21)-b-polyoxyethylene(9) (Pluronic L44) using the experimental data obtained in Examples 3 and 4.

B. CMC and partitioning coefficient of pyrene (P) were related to the structure of Pluronic block copolymers. The structure was described by variables i and j, where i equals the molecular mass of polyoxypropylene segment divided by 300 and j equals weight percentage of polyoxyethylene segments divided by 10. For convenience, $Log_{10}(CMC)$ and $Log_{10}(P)$ are used in the instead of CMC and P. Visual inspection of $Log_{10}(CMC)$ and $Log_{10}(P)$ values shows their nearly linear dependence on i and j. Therefore the following second order equations were tried:

$$Log_{10}(CMC) = (a_1*i^2+b_1*i+c_1)*(m_1*j^2+n_1*j+o_1) \quad (1)$$

$$Log_{10}(P) = (a_2*i^2+b_2*i+c_2)*(m_2*j^2+n_2*j+o_2) \quad (2)$$

The parameters a,b,c,m,n,o for each of the equations were calculated by least squares method from the experimental data of CMC and P measured for 14 pluronics. (The sum of squares of deviation ($\Sigma\delta^2$) was minimized by adjusting a,b,c,m,n,o). The calculations were performed using the Solver function in MS Excel.

C. The CMC calculations for the Pluronic base are as follows:

Pluronic	i	j	Log_{10} (CMC) From experiment	Log_{10} (CMC) Calculated	Deviation δ	δ^2	CMC Calculated	deviation %
L31	3	1	-1.9E+00	-1.96395652	-3.6E-02	1.3E-03	1.09E-02	1.9
L61	6	1	-4.0E+00	-3.78362344	1.7E-01	3.1E-02	1.65E-04	-4.4
L64	6	4	-3.3E+00	-336511356	-4.6E-02	2.1E-03	4.31E-04	1.4
F68	6	8	-3.5E+00	-3.35922878	1.6E-01	2.7E-02	4.37E-04	-4.6
L81	8	1	-4.6E+00	-4.75516172	-1.1E-01	1.3E-02	1.76E-05	2.5
P84	8	4	-3.8E+00	-4.22918915	-4.6E-01	2.1E-01	5.90E-05	12.1
P85	8	5	-4.2E+00	-4.15299367	3.3E-02	1.1E-03	7.03E-05	-0.8
F87	8	7	-4.0E+00	-4.14929574	-1.0E-01	1.1E-02	7.09E-05	2.6
L101	10	1	-5.7E+00	-5.53344161	1.4E-01	2.1E-02	2.93E-06	-2.5
L104	10	4	-5.5E+00	-4.92138283	5.6E-01	3.1E-01	1.20E-05	-10.2
F108	10	8	-4.7E+00	-4.9127765	-2.1E-01	4.6E-02	1.22E-05	4.6
L121	12	1	-6.0E+00	-6.1184631	-7.3E-02	5.3E-03	7.61E-07	1.2
L123	12	3	-5.4E+00	-5.60350968	-2.4E-01	5.6E-02	2.49E-06	4.4
F127	12	7	-5.5E+00	-5.3389579	1.8E-01	3.4E-02	4.58E-06	-3.3

D. The parameters in equation (1) are as follows : a_1 = 0.034129633; b_1 = -1.16411354; c_1= 0.41047985; m_1 = 0.003688849; n_1 = -0.04454141; o_1 = 0.748662669. Final equation for determining CMC is as follows:

$$Log_{10}(CMC) = (0.03413*i^2 - 1.1641*i - 0.4105) * (0.003689*j^2 - 0.04454*j + 0.7487) \quad (3)$$

E. The mean standard deviation of Log_{10}(CMC) is 0.24 (*i.e.*, 5.3% deviation from the original values). The CMC value predicted for Pluronic L44 is $4.69*10^{-03}$ M (Log_{10}(CMC)= -2.3291). The CMC value determined for Pluronic L44 in experiment using pyrene fluorescent probe as previously described by Kabanov *et al.* (Macromolecules 28:2303, 1995) is $6*10^{-04}$ M.

F. The P calculations for the Pluronic base are as follows:

Pluronic	i	j	Log_{10} (CMC) From experiment	Log_{10} (CMC) Calculated	Deviation δ	δ^2	CMC Calculated	deviation %
L31	3	1	2.4E+00	2.4073893	5.5E-02	3.0E-03	255.4	2.3
L61	6	1	3.6E+00	3.08349	-5.4E-01	2.9E-01	1211.9	-14.9
L64	6	4	2.6E+00	2.6903905	4.3E-02	1.8E-03	490.2	1.6
F68	6	8	2.2E+00	2.4238768	1.9E-01	3.6E-02	265.3	8.5
L81	8	1	3.4E+00	3.5537062	1.7E-01	3.0E-02	3578.5	5.1
P84	8	4	2.7E+00	3.100661	3.7E-01	1.4E-01	1260.8	13.7
P85	8	5	3.3E+00	2.9920609	-3.1E-01	9.5E-02	981.8	-9.4
F87	8	7	2.4E+00	2.8384831	4.8E-01	2.3E-01	689.4	20.3
L101	10	1	4.2E+00	4.0395083	-1.2E-01	1.3E-02	10952.3	-2.8
L104	10	4	3.8E+00	3.5245305	-2.5E-01	6.4E-02	3346.0	-6.7
F108	10	8	3.3E+00	3.1753858	-1.3E-01	1.6E-02	1497.5	-3.8
L121	12	1	4.3E+00	4.5408962	2.4E-01	5.8E-02	34745.3	5.6
L123	12	3	4.0E+00	4.1278659	1.5E-01	2.2E-02	13423.5	3.7
F127	12	7	4.0E+00	3.6269901	-3.3E-01	1.1E-01	4236.3	-8.4

D. The parameters in equation (2) are as follows: a_2 = 0.00101044; b_2 = 0.107791158; c_2 = 0.916109753; m_2 = 0.005753186; n_2 = -0.11070097; o_2 = 2.033053894. Final equation for determining P is as follows:

$$Log_{10}(P) = (0.0010104*i^2 - 0.10779*i - 0.9161) * (0.005753*j^2 - 0.1107*j + 2.033)) \quad (4)$$

E. The mean standard deviation of $Log_{10}(P)$ is 0.29 (*i.e.*, 9.3% deviation from the original values). The P value predicted for Pluronic L44 is 196.7 ($Log_{10}(P)$= -2.2937). The P value determined for Pluronic L44 in experiment using pyrene as previously described by Kabanov *et al.* (*Macromolecules,* 28:2303, 1995) is 150.

EXAMPLE 6

Combinatorial approach to identification of a biological agent composition using peptide block copolymer library

A. Goal: A procedure is designed for selection of segmented copolymers (2-20 kD) that have the following characteristics:

1) amphiphilic block copolymers capable to form micelles in aqueous solutions;

2) solubilize a given drug molecule;

3) nontoxic;

4) available for GMP chemical synthesis.

More specifically, desired macromolecules should be linear block copolymers $A^i_x(B^jA^k)_nB^l_y$ consisting of chemically joined hydrophilic A^a and hydrophobic B^b blocks, x = 0 or 1, n (0. Contained block(s) may specifically bind the drug molecule.

B. General method - iterative combinatorial approach, applying virtual and synthetic libraries:

Starting point

Step 0. Having a parent database of macromolecules. The database holds (for each entry):

(i) molecular structure type (pattern); (ii) parameters of blocks (experimental and calculated), (iii) physicochemical parameters (including data on affinity to specific drugs).

Iteration cycle

Step 1. Generation of virtual library of macromolecules. This includes: estimation of global parameters for generated molecules. The estimation is based on the data in the parent database.

Step 2. Searching the virtual library for best candidates for solubilization of the given drug.

Step 3. Chemical synthesis of library of compounds. This includes combinatorial alteration of best candidates(s) selected in step 2.

Step 4. Acquiring experimental data on the synthetic compounds. including data on the interaction with the drug(s).

Step5. Extending the parent library.

The iterations should be repeated until no improvement is obtained after last iteration.

C. Block copolymers. General formula: Block copolymers $A^{i}_{x}(B^{j}A^{k})_{n}B^{l}_{y}$ consist of chemically joined hydrophilic A^{a} and hydrophobic B^{b} blocks, $x = 0$ or 1, $n \geq 0$. The junction can be: peptide bond -CO-NH-, ester bond -CO-O-, ether bond -O-, etc. Each

block must be at least 1 kD. The block copolymer segments consist of one or more building blocks, e.g. amino acid residues, oxo acid residues, alkoxy residues etc. Peptide bond joining amino acid residues will be used for routine work.

D. Building blocks. The hydrophilic building blocks block are simple hydrophilic amino acids. The hydrophobic building blocks are amino acids with a constrained backbone including cycloaliphatic or aromatic ring. The N-terminal building block should be an acyl residue instead amino acid to keep the molecule neutral.

E. Computational methods. All computations are performed on a Windows NT workstation.

F. The database. Programmable relational database capable to cooperate with third party software. ISIS-BASE and ORACLE should be considered. MS EXCEL, VISUAL BASIC and/or VISUAL C++ can be applied for data exchange.

G. Parameters of blocks. Any QSAR parameters available, including charge, dipole moments, volume, surface, LogP and related values. These parameters may be calculated using ACD/LABS and HYPERCHEM/CHEM+ software.

H. Chemical synthesis. The macromolecules will be assembled by means of solid phase peptide synthesis, with classic Fmoc-type chemistry. All the building blocks will be introduced as Fmoc-derivatives of respective amino acid residues. To avoid charges, C-terminal amide resin and N-terminal acyl will be used. The final product should subject to very simple purification procedure, e.g. extraction on C18 cartridges.

I. Experimental data. The experimental parameters: CMC in various conditions and related parameters, partition coefficients, etc. A simple system for rapid measurement of CMC and partitioning coefficients for various small molecules must be set up.

K. The phases of the project: (i) preliminary (obtaining or synthesis and characterization of some basic building blocks, *e.g.*, H_2N-PEG1500-COOH, Fmoc-PEG1500-COOH; example syntheses; selection of the QSAR approach (parameter sets and functions); (ii) development (setting up parallel synthesis and/or synthesis of libraries; setting up the database with computational QSAR procedures; setting up assay systems; extending the set of available building blocks; application).

L. Synthesis of building blocks. Necessary materials: Rink amide AM resin; -Fmoc-PEG1500-COOH; Fmoc-NH-$(CH_2)_5$-COOH; other reagents: PyBOP, DIPEA, piperidin, TFA, triisopropylsilane, DMAP; solvents: DMF, acetonitrile.

EXAMPLE 7

Testing P-gp inhibiting effects of Pluronic copolymer compositions

A. Goal: To identify Pluronic composition having the highest activity in inhibition of glycoprotein P (P-gp) efflux system in a multiple drug resistant (MDR) cell line. The efficacy of P-gp inhibition to be characterized using the rhodamine 123 uptake test (Miller *et al.* Bioconjugate Chemistry 8: 649, 1997).

B. Materials: Rhodamine 123 was purchased from Sigma (St Louis, IL). Pluronic polymers were obtained from BASF Corp. (Parispany, NJ). The compositions of the Pluronic copolymers with rhodamine 123 were prepared to obtain a library of compositions. The following compositions are studied: 0.02%, 0.05% and 0.1% Pluronic L61, 0.02%, 0.05%, 0.1% and 0.5% Pluronic L64, 0.05%, 0.1%, 0.5%, 1%, and 2% Pluronic L44.

C. Cell line: Human breast carcinoma MCF-7 cells (ATCC HTB22) and their MDR MCF-7 ADR cell subline, derided from the parental cells by selection with Dox (Batist *et al.* 1986), were kindly presented by YL, Lee (Willian Beaumont Hospital,

Royal Oak, MI). The cells were maintained in vitro as a monolayer culture in D-MEM supplemented with 10% FBS at 37°C in a humidified atmosphere with 5% CO_2.

D. Drug uptake assay: The cells in suspension (2.5 x 10^5 cells/tube) were pre-incubated for 10 minutes at 37°C prior to drug addition. Free rhodamine 123 (0.5(M) or rhodamine 123 with Pluronic copolymers of the library of compositions were added to the cells and incubated for 45 minutes under the culture conditions as described above. The cells were then placed in an ice-water slurry to stop the incubation and washed two time with cold D-PBS. The cell fluorescence was analyzed by flow cytometry on a Coulter Epics XL cytometer, excitation 488 nm (argon laser), using a 525-nm filter. A minimum of 10,000 events were analyzed for each point. The experimental values of cell fluorescence were normalized according to the cell size by dividing the mean channel fluorescence value by the forward light scatter value. The experiments were performed in triplicates.

E. The results are presented in the table as the enhancement in rhodamine 123 uptake in the composition compared to the free rhodamine 123 uptake MCF-7 and MCF-7 Adr cells. The most active composition is Pluronic L61 (0.1%).

Composition	Increase in rhodamine 123 uptake	
	MCF-7	MCF-7 ADR
L61 (0.02%)	N/d	37
L61 (0.05%)	2.8	44
L61 (0.1%)	N/d	51
L64 (0.01%)	-1.4	2
L64 (0.02%)	-1.3	3.3
L64 (0.05%)	1	11.5
L64 (0.1%)	1.1	31
L64 (0.5%)	1.3	40
L44 (0.05%)	1.3	1.5
L44 (0.1%)	-1.3	2.6
L44 (0.5%)	1	29.5
L44 (1%)	1.4	26
L44 (2%)	1.6	25

EXAMPLE 8

Testing P-gp inhibiting effects of Pluronic copolymer compositions

A. Goal: To identify Pluronic composition having the highest activity in inhibition of glycoprotein P (P-gp) efflux system in a multiple drug resistant (MDR) cell line. The efficacy of P-gp inhibition to be characterized using the rhodamine 123 uptake test (Miller *et al.* Bioconjugate Chemistry 8: 649, 1997).

B. Compared to Example 7, the broader base of Pluronic copolymers is used including Pluronic L31, polyoxyethylene(41)-b-polyoxypropylene(16)-b-polyoxyethylene(41) (Pluronic F38), Pluronic L44, Pluronic L61, Pluronic L64, Pluronic F68, Pluronic L81, Pluronic P84, Pluronic P85, Pluronic F87,

polyoxyethylene(123)-b-polyoxypropylene(48)-b-polyoxyethylene(123) (Pluronic 98), Pluronic L101, Pluronic L104, polyoxyethylene(34)-b-polyoxypropylene(53)-b-polyoxyethylene(34) (Pluronic 105), Pluronic F108, Pluronic L121, Pluronic L123 and Pluronic L127. The concentration of Pluronic copolymers are 0.0001%, 0.001%, 0.01%, 0.1% and 1%.

C. The drug uptake test is performed as described in example 7.

D. The results are presented in the table as the enhancement in rhodamine 123 uptake in the composition at the optimal Pluronic concentration compared to the free rhodamine 123 uptake in MCF-7 Adr cells. The most active composition is Pluronic L61 - i.e., the result is the same as in example 7 using a narrower library of composition. This demonstrates that the desired biological agent composition can be identified using very limited number of compositions in the library.

Composition	Increase in rhodamine 123 uptake
L31	60
L38	14
L44	12
L61	90
L64	60
L68	35
L81	60
P84	40
P85	65
F87	14
F98	17
L101	43
L104	60
P105	14
F108	18
L121	18
L123	30
F127	12

EXAMPLE 9

Testing anti-MDR activity of Pluronic copolymer compositions

A. Goal: To identify composition of Doxorubicin (Dox) having the highest cytotoxic activity against an MDR cell line.

B. Materials: Dox was purchased from Sigma (St Louis, IL). Pluronic polymers were obtained from BASF Corp. (Parispany, NJ). The compositions of the Pluronic copolymers with Dox were prepared to obtain a library of compositions. The following compositions are studied: 0.02%, 0.025% and 0.1% Pluronic L61, 0.02%, 0.05% and 0.1% Pluronic L64, 0.02%, 0.05%, 0.1% and 0.5% Pluronic L44, 0.008%, 0.04%, 0.2% and 1% Pluronic P85, 1% Pluronic F108, 0.2 % Pluronic F127, mixture of 0.025 % Pluronic L61 and 0.2 % Pluronic F127.

C. Cytotoxicity assay: The cells were placed (3000 cells per well) in a 96-well plate and allowed to reattaching overnight. Dox or Dox-formulated with polymers was incubated with the cells for 2 h at 37°C with 5% CO_2. The cells were washed three times and cultured for 4 days. The drug cytotoxicity was determined by a standard 2,3-bis[2-methoxy-4-nitro-5-sulfophenyl]-2H-tetrazolium-carboxanilide inner salt (XTT) assay (Scudievo *et al.*, 1988). The absorbance at (450 was determined using a microplate reader. All the experiments were carried out in triplicates. SEM values were less than 10% ($p < 0.05$).

D. The results are presented in the table as the resistance reversion index, i.e. ratio of IC_{50} in free drug to IC_{50} of the drug-copolymer composition. The most active composition is Pluronic L61 (0.1 %).

Composition	Increase in rhodamine 123 uptake	
	MCF-7	MCF-7 ADR
L61 (0.02%)	N/d	37
L61 (0.05%)	2.8	44
L61 (0.1%)	N/d	51
L64 (0.01%)	-1.4	2
L64 (0.02%)	-1.3	3.3
L64 (0.05%)	1	11.5
L64 (0.1%)	1.1	31
L64 (0.5%)	1.3	40
L44 (0.05%)	1.3	1.5
L44 (0.1%)	-1.3	2.6
L44 (0.5%)	1	29.5
L44 (1%)	1.4	26
L44 (2%)	1.6	25

Composition	Resistance reversion index	
	MCF-7	MCF-7 ADR
L61 (0.02%)	1	125
L61 (0.1%)	1.1	740
L64 (0.02%)	1	40
L64 (0.05%)	1	72
L64 (0.1%)	1	529
L44 (0.02%)	1	3.3
L44 (0.05%)	-1.4	4
L44 (0.1%)	-1.3	11.8
L44 (0.5%)	-1.3	25
P85 (0.008%)	1	2.8
P85 (0.04%)	1	6.4
P85 (0.2%)	1	9.3
P85 (1%)	1	9.4
F108 (1%)	1	3
L61 (0.025%)	1.9	115
F127 (0.2%)	1	1.3
L61/F127 (0.025%/0.2%)	1.3	129

What is Claimed

1. A method of identifying a desired biological agent composition comprising:

(a) preparing a plurality of segmented copolymers, said segmented copolymers differing in at least one of the following, (*i*) at least one of their segment lengths, (ii) chemical structure, or (iii) copolymer architecture,

(b) preparing at least one composition of said segmented copolymers with at least one biological agent;

(c) testing at least some said compositions of said segmented copolymers with a biological agent for biological properties; and

(d) identifying said compositions with desired biological properties.

2. The method according to claim 1 wherein the segmented copolymers are block copolymers.

3. The method according to claim 1 wherein the segments contain at least three repeating units.

4. The method according to claim 1 wherein the segments contain at least between about 5 and about 200 repeating units.

5. The method according to claim 1 wherein said segmented copolymer has at least one hydrophilic nonionic polymer and at least one hydrophobic nonionic segment.

6. The method according to claim 1 wherein said segmented polymers have at least one cationic segment and at least one nonionic segment.

7. The method according to claim 1 wherein said segmented polymers have at least one anionic segment and at least one nonionic segment.

8. The method according to claim 1 wherein said segmented polymers have at least one polynucleotide segment and at least one segment which is not a nucleic acid.

9. The method according to claim 1 wherein the compositions are tested in a biological model.

10. The method according to claim 2 wherein the compositions biological model is a cell model.

11. The method according to claim 2 wherein the biological model is an animal model.

12. The method according to claim 2 wherein the wherein the biological model is a plant model.

13. The method according to claim 1 wherein said polymer segments comprise at least one polypeptide segment and at least one non-peptide polymer segment.

FIGURE 1

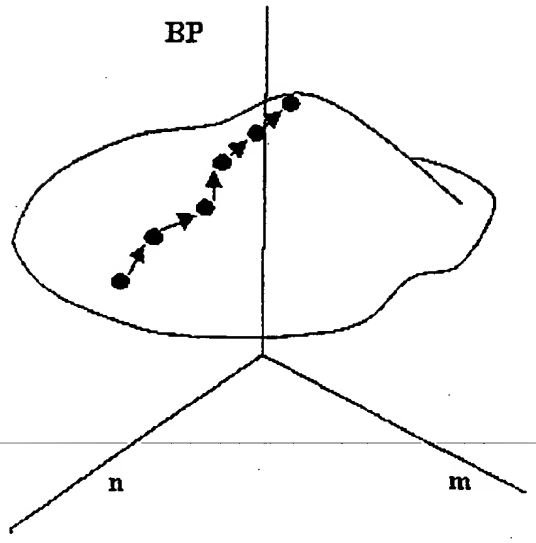


FIGURE 2

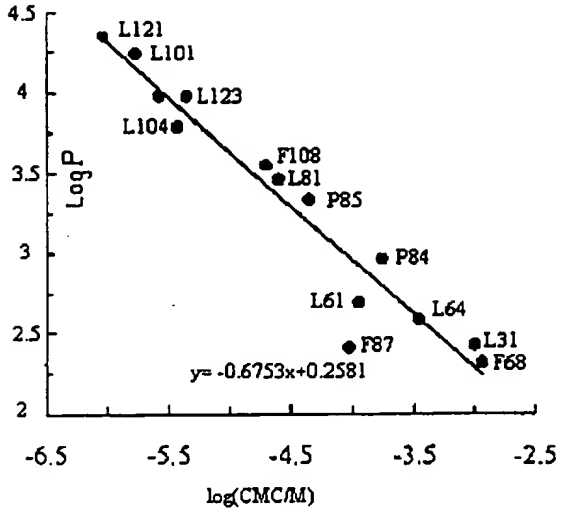

4.5
4
3.5
3
2.5
2
Log P
L121
L101
L123
L104
F108
L81
P85
P84
L61
L64
L31
F68
F87
y= -0.6753x+0.2581
-6.5
-5.5
-4.5
-3.5
-2.5
log(CMC/M)

FIGURE 3

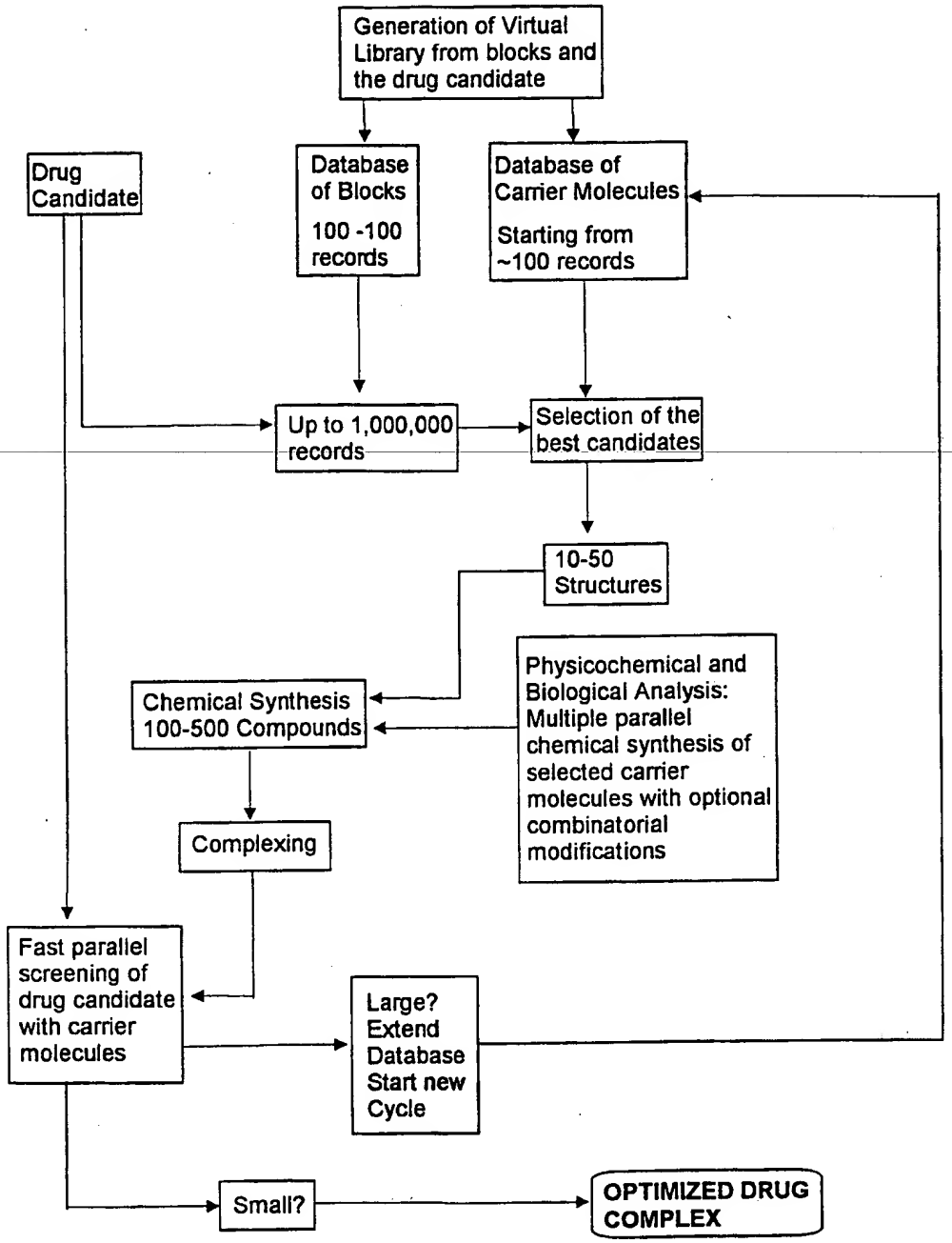
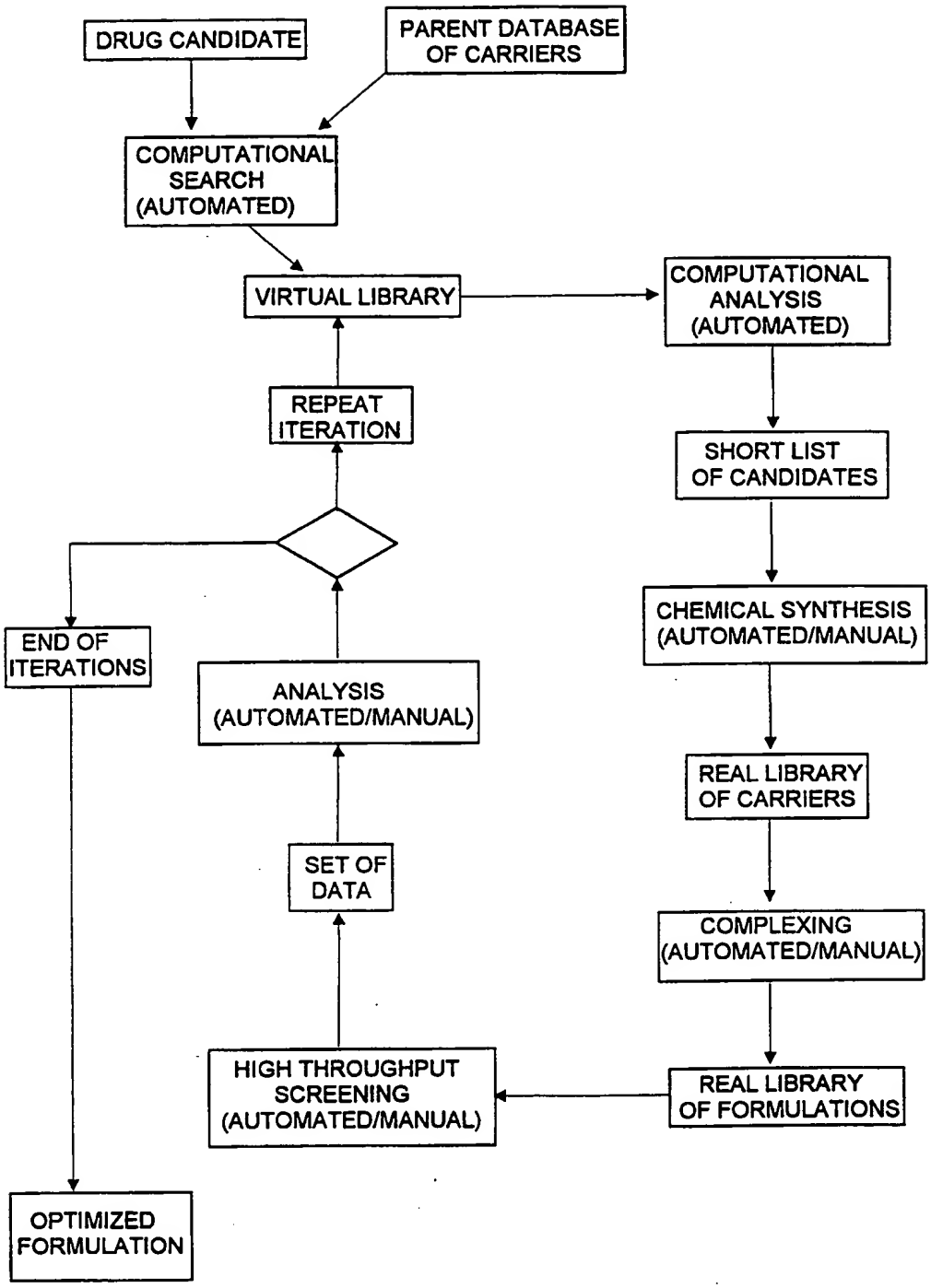

Generation of Virtual Library from blocks and the drug candidate
Drug Candidate
Database of Blocks
100 -100 records
Database of Carrier Molecules
Starting from ~100 records
Up to 1,000,000 records
Selection of the best candidates
10-50 Structures
Chemical Synthesis 100-500 Compounds
Physicochemical and Biological Analysis: Multiple parallel chemical synthesis of selected carrier molecules with optional combinatorial modifications
Complexing
Fast parallel screening of drug candidate with carrier molecules
Large? Extend Database Start new Cycle
Small?
OPTIMIZED DRUG COMPLEX

FIGURE 4


DRUG CANDIDATE
PARENT DATABASE OF CARRIERS
COMPUTATIONAL SEARCH (AUTOMATED)
VIRTUAL LIBRARY
COMPUTATIONAL ANALYSIS (AUTOMATED)
REPEAT ITERATION
SHORT LIST OF CANDIDATES
CHEMICAL SYNTHESIS (AUTOMATED/MANUAL)
END OF ITERATIONS
ANALYSIS (AUTOMATED/MANUAL)
REAL LIBRARY OF CARRIERS
SET OF DATA
COMPLEXING (AUTOMATED/MANUAL)
HIGH THROUGHPUT SCREENING (AUTOMATED/MANUAL)
REAL LIBRARY OF FORMULATIONS
OPTIMIZED FORMULATION

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US98/16300

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) :G01N 33/543
US CL : 436/532, 523, 518

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 436/532, 523, 518

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

APS, CAS, BIOSIS
SEARCH TERMS: COMBINATORIAL LIBRARY, SEGMENTED COPOLYMERS, CARRIER, SURFACTANT

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	KABANOV A.V. et al. Micelle Formation and Solubilization of Fluorescent Probes in Poly(oxyethylene-b-oxypropylene-b-oxyethylene)Solutions, Macromolecules. (1995). Vol. 28. pages 2303-2314, entire document.	1-13
Y	VINOGRADOV S. V. et al. Block polycationic Oligonucleotide Derivative: Synthesis and Inhibition of Herpes Virus Reproduction, Bioconjugate Chem. (1996). Vol. 7, pages 3-6, especially page 3.	1-4, 6, 8, 9-11
Y	KABANOV A.V. et al. The neuroleptic activity of haloperidol increases after its solubilization in surfactant micelles. FEBS. December 1989. Vol. 258. No. 2. pages 343-345, especially pages 343 and 344.	1-7, 9-11, 13

[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E"	earlier document published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search 01 DECEMBER 1998	Date of mailing of the international search report 20 JAN 1999
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703) 305-3230	Authorized officer T. WESSENDORF Telephone No. (703) 308-0196

Form PCT/ISA/210 (second sheet)(July 1992)*

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US98/16300

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X ---- Y	WOIFERT M.A. et al. Characterization of Vectors for Gene Therapy formed by Self-Assembly of DNA with Synthetic Block Co-Polymers. Human Gene Therapy. 10 November 1996. Vol. 7. pages 2123-2133, especially pages 2123-2124, 2130-2131.	1-8, 9-11 ---------- 12-13
X ----- Y	KATAYOSE S. et al. Water Soluble Polyion Complex Between DNA and Peg-Poly(L-Lysine)Block Copolymer for Novel Gene Vector. Proceed. Intern. Symp. Control. Rel. Bioact. Mater. 1996. Vol. 23. pages 899-900. See entire document.	1-8 ------ 9-13
Y	US 5,624,711 A (SUNDBERG et al) 29 April 1997, col. 13, line 45 to col. 16, line 45.	1-11, 14